GENETICS

A periodical record of investigations bearing on heredity and variation

Founded in 1916 by GEORGE H. SHULL

with the cooperation of

WILLIAM E. CASTLE	BRADLEY M. DAVIS	HERBERT S. JENNINGS
EDWIN G. CONKLIN	EDWARD M. EAST	THOMAS H. MORGAN
CHARLES B. DAVENPORT	ROLLINS A. EMERSON	RAYMOND PEARL

VOLUME 40 **SEPTEMBER, 1955** **NUMBER 5**

TABLE OF CONTENTS

Abstracts of Papers Presented at the 1955 Meetings of the Genetics Society of America 563

WHITE, M. J. D., and F. H. W. MORLEY, Effects of pericentric rearrangements on recombination in grasshopper chromosomes 604

NADEL, ELI M., JOSEPH GREENBERG, GEORGE E. JAY, and G. ROBERT COATNEY, Backcross studies on the genetics of resistance to malaria in mice .. 620

ALLEN, SALLY LYMAN, Linkage relations of the genes histocompatibility-2 and fused tail, brachyury and kinky tail in the mouse, as determined by tumor transplantation 627

PRICE, SAM, Irradiation and interspecific hybridization in Secale 651

NANNEY, D. L., P. A. CAUGHEY, and A. TEFANKJIAN, The genetic control of mating type potentialities in *Tetrahymena pyriformis* 668

KEMPTHORNE, OSCAR, The correlations between relatives in inbred populations 681

OSTER, I. I., Modification of X-ray mutagenesis in Drosophila. I. Reunion of chromosomes irradiated during spermiogenesis 692

EMMERLING, M. H., A comparison of X-ray and ultraviolet effects on chromosomes of *Zea mays* 697

JENKINS, J. A., AND G. MACKINNEY, Carotenoids of the apricot tomato and its hybrids with yellow and tangerine 715

LOCKHART, WILLIAM R., and HAROLD R. GARNER, Genetic mechanisms governing the effect of canavanine on *Neurospora crassa* 721

RYAN, FRANCIS J., Spontaneous mutation in non-dividing bacteria 726

JUDD, BURKE H., Direct proof of a variegated-type position effect at the white locus in *Drosophila melanogaster* 739

ROBERTSON, DONALD S., The genetics of vivipary in maize 745

PUBLISHED BIMONTHLY AT BALTIMORE, MARYLAND
BY GENETICS, INC.

(Date of issue, September 30, 1955)

EDITORIAL BOARD

R. ALEXANDER BRINK, *Managing Editor*,
JAMES F. CROW, *Associate Managing Editor*,
University of Wisconsin

GEORGE W. BEADLE
California Institute of Technology

WILLIAM E. CASTLE
University of California

BRADLEY M. DAVIS
University of Michigan

EVERETT R. DEMPSTER
University of California

L. C. DUNN
Columbia University

JOHN W. GOWEN
Iowa State College

A. D. HERSHEY
Carnegie Institution of Washington

DONALD F. JONES
Connecticut Agricultural Experiment Station

M. M. RHOADES
University of Illinois

T. M. SONNEBORN
Indiana University (Representative of the Genetics Society of America)

CURT STERN
University of California

ALFRED H. STURTEVANT
California Institute of Technology

SEWALL WRIGHT
University of Wisconsin

Volume 40 SEPTEMBER, 1955 Number 5

GENETICS is a bimonthly journal issued in annual volumes of about 600 pages each.

Subscription, $8.00 net a year for complete volumes (January–November). Foreign postage, 50 cents additional. Single copies, $1.50 each, postpaid.

As available, back volumes may be had at $14.00 each and single issues of previous volumes at $2.50 per copy, postpaid. The Business Office will supply information on request as to volumes and numbers available.

Business Correspondence, including change of address, etc., should be addressed to GENETICS, INC., Business Office, Mount Royal and Guilford Avenues, Baltimore 2, Maryland.

Remittances should be made payable to GENETICS, INC.

Correspondence concerning editorial matters should be addressed to the EDITORS OF GENETICS, Genetics Building, University of Wisconsin, Madison 6, Wisconsin.

Entered as second-class matter at the postoffice at Baltimore, Md., under Act of March 3, 1879. Acceptance for mailing at the special rate of postage provided for in the Act of February 28, 1925, paragraph 4, section 412, P.L.&R., authorized January 9, 1932.

Claims for missing numbers should be made within 30 days following their date of mailing. The publishers will supply missing numbers free only when they have been lost in the mails.

ABSTRACTS OF PAPERS PRESENTED AT THE 1955 MEETINGS OF THE GENETICS SOCIETY OF AMERICA

EAST LANSING, MICHIGAN, SEPTEMBER 6–8, 1955

ALTENBURG, LUOLIN S., Rice Institute, Houston, Texas: *The non-effect of photoreactivating light on the mutation rate induced in Drosophila by tertiary-butyl hydroperoxide.*—The polar cap ends of dechorionated fertilized eggs of *D. melanogaster* (containing the cells of the early germ track) were exposed for 20 minutes to the vapor of a 0.5M solution of tertiary-butyl hydroperoxide (dissolved in di-butyl phthalate). The eggs were divided into two groups, one group being subjected to posttreatment for an additional 20 minutes at a distance of 50 cm with the light from a 100-watt General Electric CH-4 spotlight lamp, a large percent of the output of which was radiation ranging in wavelength from 3000 to 4200A. The other group of eggs was placed at the same distance from the lamp for the same length of time but was shielded as much as possible from its radiation. Both groups were then allowed to develop in darkness. A third group of eggs was exposed to the vapor of di-butyl phthalate (in which the peroxide used in the treated series was dissolved) for 20 minutes, then placed in front of the CH-4 lamp but shielded from the radiation. Lethal mutations in the second pair of chromosomes of the males that had been treated in the egg stage were detected by MULLER'S *sifter* technique. The mutation rate induced in the eggs by treatment with tertiary-butyl hydroperoxide alone was 6.6 ± 2.0%; that of the eggs exposed to posttreatment by long wavelength ultraviolet and visible light was 5.7 ± 2.2%. No mutations were recovered from the eggs exposed to the vapor of di-butyl phthalate but shielded from the posttreatment. These results indicate that the reduction in mutation rate caused by photoreactivating light when applied to ultraviolet-treated material occurs before the stage of peroxide production; or that mutagenesis is a branched process, one or more branches of which do not involve peroxide production, and that photoreactivating light does not influence these branches. (This work has been supported by a grant received for work of EDGAR ALTENBURG and associates from the National Institutes of Health, U. S. Public Health Service.)

ANDERSON, LULU F., Brookhaven National Laboratory, Upton, N. Y.: *Effects of centrifugation on X-ray-induced chromatid aberrations in Tradescantia irradiated in air and N_2.*—Inflorescences of Tradescantia were irradiated with 100r of X-rays given in 1 minute in air and centrifuged immediately. After 19 hours fixations were made for chromatid break studies. Five hundred cells from at least three slides per treatment were scored. An increase of almost 100% in isochromatid and chromatid breaks was observed in microspore metaphase over values obtained from irradiated, non-centrifuged samples. Smaller differences were observed for total exchanges. This was partly due to a decrease in the number of rings with centrifugation while interchanges and triradials increased. It appeared that total breakage was increased and that the ratio of intrachromosomal (interchanges and triradials) to interchromosomal (rings) exchanges had increased. This implied an increase in randomness in the rejoining process. Similar treatments in a nitrogen atmosphere with a dose of 200r given in 1 minute showed smaller differences between centrifuged and non-centrifuged samples. Chromatid and total exchange values showed little if any increase with centrifugation while isochromatid values showed a definite increase. Samples centrifuged 30 seconds after irradiation in N_2 showed a slight increase in all classes of breaks over those centrifuged 2½ minutes after irradiation. Restitution in nitrogen would, therefore, seem to begin immediately after breakage. (Research carried out at Brookhaven National Laboratory under the auspices of the U. S. Atomic Energy Commission.)

ATWOOD, K. C. and THAD H. PITTENGER, Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee: *The relation between the X-ray survival curves of Neurospora microconidia and ascospores.*—The survival curve of uninucleate microconidia of *Neurospora crassa* with X-rays (250 kvp with 3 mm of Al filtration) is complex, having both single and multiple target components. The survival curve of the ascospores, which are binucleate, was found to be related in a simple way to that of the uninucleate microconidia. The ascospore survival is given by $S_{asc.} = 1 - (1 - s)^2$ where s is the survival of microconidia. In other words, the ascospores behave as though each consisted of

Second Printing 1970 / University of Texas Printing Division, Austin

two independent microconidia taken together. The mutual independence of the nuclei in ascospores is in contrast to the nuclear interaction in multinucleate macroconidia in which the survival curve cannot be derived from that of microconidia.

BEATTY, ALVIN V., Emory University, Ga.: *The effects of low intensity X-radiation on the production of chromosomal aberrations in Tradescantia microspores.*—The frequency of chromosomal aberrations observable during the first microspore division in *Tradescantia paludosa* was used as a measure of the effects of low intensity X radiation in atmospheres of helium and pure oxygen. A total dose of 400r was used with deliveries at intensities of 1r, 2r, 3r, 5r, 15r and 25r per minute. The aberration frequency in helium was highest at 1r, 68.5%, and lowest at 25r, 24.5%. In oxygen the lowest frequency, 31.1%, was with 1r per minute while the highest, 76.8%, was at 25r. At 2r per minute the aberration frequency was about the same in both helium and oxygen.

BECKER, W., University of California, Berkeley, Calif.: *Maternal influence in rat lines selected for body weight.*—Two lines of rats selected for large body weight were reciprocally crossed with two lines selected for small body weight. These lines were originally derived from the same F_2, and selection was based upon the individual's 70-day weight within its litter. A double first cousin mating system was utilized. When reciprocally crossed the lines were somewhat inbred, and the two Large lines differed significantly in weight when compared with the Small lines. The mean 28-day weaning weight of the cross Large female times Small male approximated the 28-day means of the Large lines, while the reciprocal cross approximated those of the Small lines. The significant difference between reciprocal crosses indicates that rat mothers strongly influence their offspring's weaning weight. At 70 days the difference was evident but not significant. These striking differences between reciprocal crosses of lines selected only for individual 70-day weight indicate a positive genetic correlation between maternal influence and individual growth during selection in these lines. No evidence for 'compensatory growth' after weaning was found. The within litter variance at weaning of the cross Large female times Small male was significantly less than the variance of the reciprocal cross suggesting that maternal environment may affect variability of early growth in rats. At 70 days both crosses showed less variability than their parental lines.

VON BORSTEL, R. C., K. C. ATWOOD, and A. R. WHITING, Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tenn., and University of Pennsylvania, Philadelphia, Pa.: *Delayed expression of induced dominant lethals in diploid Habrobracon.*—In Habrobracon unfertilized eggs develop normally to become haploid males; fertilized eggs become diploid females. Females which had stored eggs in the first meiotic metaphase were X-irradiated and one half of them subsequently mated. The fertilized eggs had a much higher hatchability frequency than the unfertilized eggs; however, adult survival did not differ markedly in the two groups. The higher frequency of death during the larval stage in the diploid embryos accounts for the difference. Those mutations which cause death of diploid embryos at a later stage of development than haploid embryos are referred to as conditionally delayed dominant lethal mutations; up to 25% or more of dominant lethals can be of this class in eggs irradiated in metaphase I. Examination of unfixed dead embryos also shows that fertilized eggs die at later stages than do unfertilized ones when the eggs are irradiated in either the first meiotic metaphase or prophase, but a higher proportion of eggs, fertilized and unfertilized, die early in the latter case. Conditionally delayed dominant lethals by criterion of hatchability do not occur to any appreciable extent when eggs are irradiated in the first meiotic prophase.

BRYAN, JOHN H. D., and JOHN W. GOWEN, Iowa State College, Ames, Iowa: *A cytological and photometric study of the effects of X-rays on the mouse testis.*—Forty-day males of strains BALB/Gw and S were given a dose of either 320 or 2560r (250 pkv, 30 ma; filtration 0.25 mm Cu, 1 mm Al; dose rate 430r/min.). The irradiation was delivered to the pelvic region, the rest of the body being shielded. Control and experimental animals were sacrificed at 1, 8 and 24 hours, and at intervals up to 28 days after irradiation. The DNA (Feulgen) content of spermatogonial nuclei was estimated by means of a cytophotometer. The patterns of response to irradiation were found to be essentially iden-

tical for the strains used. Cellular changes were more rapid and pronounced at the higher dosage level; the results reported here are based mainly on data from strain BALB/Gw following 320r. No change in the number of "resting" spermatogonia was evident at 24 hours after irradiation; by 3 days, the level had been reduced to less than 5% of the control value. Regeneration of spermatogonia commenced at about 10 days and at 28 days had reached a level exceeding 1.5 times that of the corresponding controls. Other spermatogenic elements behaved in a similar manner, the time relationship being close to that of normal spermatogenesis. The photometric data together with the cytological studies support the conclusion that radiation-induced inhibition of spermatogonial DNA synthesis is primarily responsible for the cellular changes observed in the mouse testis. (This work has received assistance from contract No. AT(11-1)107 from the Atomic Energy Commission.)

BUTLER, L., University of Toronto, Toronto, Canada: *A recessive lethal closely linked with wooly in the tomato.*—From 1930–53 all F_2 segregations involving the dominant mutant wooly *Wo* gave 2:1 ratios (3032:1597) and no *Wo Wo* plants were produced. As a result, wooly was classified as lethal when homozygous. In 1954, one F_2 gave a 3:1 ratio (1278:452) and homozygous *Wo Wo* were isolated. At the same time, Wo^m a multiple allele with no lethal effect was found by C. M. RICK, so mutation in this cross was suspected. This hypothesis was abandoned when the cross of a *d m* selection from this F_2 with *aw* gave high heterogeneity in the monohybrid ratios. Some F_1 plants yielded F_2's in the expected segregations while the rest of the F_1's gave an excess of *aw* and a deficiency of *d*, a typical ratio being ++ 518: + *aw* 303:*d* + 135:*d aw* 17. This ratio can be best interpreted by postulating a lethal *le* near the *Wo* locus. In the past *le* has always been linked with *Wo* but a rare crossover from + *Wo le*/*d* ++ gave *d* + *le*/*d* ++ which crossed with *aw aw* gave the two F_1's *d* ++/+ *aw* + and *d* + *le*/+ *aw* + . The linear order for genes tested with this chromosome 2 (1) lethal is *d m dv ro aw Wo le.*

BUTZEL, H. M., JR. and W. B. MARTIN, JR., Union College, Schenectady 8, New York: *Studies of amino acid constituents of Paramecium aurelia.* Different stocks, mating types, and serotypes of *P. aurelia* have been analysed for qualitative content of amino acids. After acid hydrolysis and two-dimensional paper chromatography (butanol-acetic acid; cresol-phenol) using buffered Whatman #1 papers, the following 13 amino acids were identified in all stocks: alanine, arginine, aspartic acid, glutamic acid, glycine, isoleucine, leucine, lysine, phenylalanine, serine, threonine, tyrosine, and valine. No qualitative differences were found between stocks, mating types, or serotypes, or between hydrolysates of entire animals, breis of animals, or supernatants of weakly centrifuged breis. Similarly prepared chromatograms of *Aerobacter aerogenes*, the bacteria with which the paramecia cultures were fed, revealed the same 13 amino acids, and, in addition, cystine and proline. Further studies are being carried out to determine whether differences exist in quantitative content of amino acids or in structural configuration of the proteins in the various stocks analysed. (Work supported by research grants from the National Science Foundation, and from the National Cancer Institute, National Institutes of Health, Public Health Service.)

CALDECOTT, RICHARD S., U. S. Department of Agriculture, University of Minnesota, St. Paul, Minnesota and the Brookhaven National Laboratory, Upton, New York: *Protection from X-ray induced injury by hydration.*—Prior to X-radiation the water content of seeds of barley has been modified by (1) storage at different relative humidities, (2) steeping for 16 hours at 0°C followed by various periods of desiccation and (3) steeping at 0°C and 22°C for different periods of time. Pooling the data from all studies clearly shows that the sensitivity of barley seeds to X-rays, as measured by inhibition of seedling growth and the frequency of chromosomal aberrations, decreases as the water content of the embryo increases from about 4 percent to about 8 percent. At this upper level a plateau is reached and further addition of water to the embryo (up to 60 percent) results in no additional modification of sensitivity, providing cellular activity is inhibited by low temperature. The data suggest that previous ideas concerning the effect of hydration in substantiating the indirect action hypothesis of the action of radiation in cellular systems should be modified. (Research carried out at Brookhaven National Laboratory under the auspices of the U. S. Atomic Energy Commission.)

CALE, GLADSTONE H., JR., Dadant and Sons, Hamilton, Illinois, and JOHN W. GOWEN, Iowa State College, Ames, Iowa: *Heterosis in honey bees (Apis mellifera L.).*—Two honey bee characteristics, oviposition rate and honey yield, were studied in four inbred lines, their six nonreciprocal F_1's, and a random check stock. Heterotic effects were demonstrated for both oviposition rate and honey yield. These effects were such that five of the six F_1's exceeded their higher parent in oviposition rate, and four of the random mating F_1's exceeded their higher parent for honey yield. Expressed as a percentage of the higher parent, the egg productivities of the F_1's ranged from 128 percent to 166 percent, with an average productivity increase of 35.5 percent. F_1 honey yield increases ranged from 117 percent to 129 percent of the higher parent, with an average yield increase over the higher inbred parent of 14.7 percent. The oviposition rate of the average of the hybrids was 107.2 percent of the random stock. The two better hybrids exhibited productivities of 114.4 and 116.7 percent of the random stock. Expressed in terms of the random stock, the average honey yield of the hybrids was 106.2 percent. The better yielding hybrid produced at the rate of 123.7 percent of the random stock. Under the conditions of this experiment, inbreds were shown to exhibit both general and specific combining ability differences for oviposition rate and honey yield. The contributions of general combining ability to high yield and high oviposition rate were of greater importance than were the contributions of specific combining ability.

CASPARI, ERNST and WILLIAM F. ARNDT, Department of Biology, Wesleyan University, Middletown, Conn.: *Mutation patterns in the alga Scenedesmus.*—Cultures of the alga *Scenedesmus quadricauda* were irradiated with UV and plated out on agar. The resulting plates were scored for colonies showing abnormal chlorophyll coloration. It appeared that about 40% of the mutant colonies were complete, while the remainder contained both mutant and normal green parts. The mutants derived from complete colonies were stable on subculture. The organisms from mixed cultures behaved differently from each other: out of 44 colonies tested, 16 proved to be stable on subculture, 26 reverted gradually to the normal phenotype, and 2 continued to give rise to mixed colonies. Reversion is a gradual process in which all organisms in one culture at a particular time are affected to the same degree; it does not appear to be due to backmutation and selection. It is proposed that the stable mutations are true gene mutations, whereas the reversible mutations may represent plastid mutations. There is a definite correlation between the appearance of the mutant part of the original colony and the behavior in subculture: the reverting type appears as half colonies, quarter colonies or sectors; the stable type appears as mutant areas on the periphery, as secondary colonies, or as a light mantle around a normal green core; the eversporting colonies appear mottled. If the organisms are permitted to divide after irradiation, the number of mixed colonies decreases while the number of complete mutant colonies increases. This is in part due to the fact that the mixed colonies segregate out. This is borne out by the finding that some of the complete mutant colonies found at 72 hours revert to normal. But the number of mutants found after the delay is higher than can be accounted for by segregation of mixed mutant clones. The delay in the expression of mutations in Scenedesmus is considered a complex phenomenon.

CHAI, C. K., Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine: *Genetics of body size in mice: A quantitative approach to gene action and segregation.*—A study has been made of the genetics of body size in mice by crossing Large and Small strains of mice to produce F_1 and F_2 backcross generations. 60-day body weight was taken as the criterion. The F_1 and F_2 means were close to the midpoint between parental strain means. All backcross means were also close to the midpoints between means of their respective parents. Means of F_2 and backcross generations were slightly above the theoretical midpoint values. The variance of the F_2 hybrids was largest, of backcross generations next, and the average of the variances of F_1 hybrids and parental strains smallest. The effective size genes involved in this cross have been found to act additively on an arithmetic scale rather than on a logarithmic scale. The computed value of additive genetic variance is 5.63 and of dominance variance is .91. The ratio of dominance to additive genetic variance is .16. The number of segregating units has been estimated at 13 or more. Each unit has been assumed to include at least one locus modifying body size.

CHAO, P. K., Indiana University, Bloomington, Ind.: *Hereditary modification of the action of macronuclear k genes in the maintenance of the killer trait in variety 4 of Paramecium aurelia.*—Ordinarily kappa and the killer trait are lost by the 15th fission after *Kk* becomes *kk* at autogamy. Under certain conditions not yet thoroughly identified (of which early brief exposure to low temperature is regularly effective, though not always necessary), kappa and the killer trait may be maintained permanently throughout the life of the *kk* clone. However, they are invariably lost at the next (second) autogamy, regardless of conditions, so long as a new macronucleus arises from a product of the syncaryon. On the contrary, they are maintained if the new macronucleus arises from a piece of the old macronucleus; this has been demonstrated through three successive macronuclear regenerations. The effect is thus carried hereditarily only by the *kk* macronucleus that arises in a cell which previously was *Kk*; and neither descendants of this macronucleus nor the cells which contain them can transmit the effect to an independently produced macronucleus. Two other differences distinguish the action of the modified *kk* macronuclei from *Kk* macronuclei: (1) the *kk* killers become sensitives when the food supply declines; (2) they sometimes show no kappa in the cytoplasm even when they are killers. Because of these differences, it seems unlikely that *k* has been transduced to *K*. Possibly in the *kk* killers kappa is maintained in the macronucleus or only the "bright" spot (shown by PREER *et al.* to be the basis of the killing trait) is maintained. (Work supported by an American Cancer Society Grant to T. M. SONNEBORN.)

CHU, ERNEST H. Y., Yale University, New Haven, Connecticut: *The mechanism of transfer of a gene from Nicotiana glutinosa to N. tabacum.*—The mechanism of incorporation of chromosome material from *Nicotiana glutinosa* into *N. tabacum* and the effects of a specific *glutinosa* allele on segregation ratios in *tabacum* have been investigated. Since the F_1 *glutinosa-tabacum* hybrid is sterile, it was necessary to use the sesquidiploid obtained from 4n *N. tabacum* × 2n *N. glutinosa*. This 3n *tabacum-tabacum-glutinosa* hybrid as well as subsequent selfed generations were studied in detail, using *tabacum* plants genetically white (*ws, ws*) and selecting for the Ws_g factor carried on an extra *glutinosa* chromosome. A chromosome constitution of 24 *tabacum* bivalents + 1 *glutinosa* univalent (Ws_g) was obtained early in the program, and the transmission rates of the Ws_g chromosome were analysed. Pollen and ovule fertility was found to be reasonably high in the sesquidiploid and later generations, but no well defined correlation between fertility and number of alien chromosomes could be established. There were apparently qualitative differences between individual members of the *glutinosa* genome in this regard. Alien addition types with twenty-five pairs were obtained but were unsuitable because of their instability and the undesirable *glutinosa* features introduced. The mechanism of transfer depends primarily on partial homology between chromosomes of the two species. Infrequent trivalent formation or non-conjugation of *tabacum* homologues may result in chance substitution of a *glutinosa* chromosome for one of the *tabacum* complement. Rare association and exchange of segments between *tabacum* and *glutinosa* chromosomes may even lead to incorporation of products of segmental exchange into *tabacum*. Products of these types of behavior were distinguished by segregation ratios of green and white seedlings. Plants having twenty-four pairs and including the Ws_g segment were closely similar to *N. tabacum* when the segment was present in single dose. Homozygous Ws_gWs_g individuals were characterized by various abnormal features presumably due to the homozygous deficiency in the modified *tabacum* chromosomes.

CLANCY, C. W., University of Oregon, Eugene, Oregon: *An anomalous pigmentation of the Malpighian tubules in Drosophila melanogaster associated with a variegated-type position effect on the eyes.*—In the course of transplantation experiments involving strains heterozygous for *In(2LR)40d* of HINTON and ATWOOD, a previously undescribed character was found which is directly related to the eye abnormalities inherited as dominant effects of this chromosomal rearrangement. The new trait is a deep red coloration of the Malpighian tubules that occurs only after eclosion, and is dependent upon the attainment of a characteristic threshold level of eye defect. When genetic and culture conditions are such that all flies show some disintegration of ommatidial structure, then all eventually develop red Malpighian tubules. The latter are readily discernible, especially through the ventral abdominal wall. Microscopic examination of living tubules shows the red pigment to be located in cytoplasmic spherules similar in size and distribution to those colored yellow in wild type. Absorption spectra of

tubule extracts, and results from comparative paper chromatography indicate that the red pigment from the tubes is the same as that from the eyes. Developmental studies suggest that the "red" pigment component of wild type eye color is absorbed by the tubules from the haemolymph in these inversion heterozygotes as the eye tissues are progressively degraded in the imago. (Work supported by a grant from the American Cancer Society, on recommendation of the Committee on Growth of the National Research Council.)

COE, E. H., Field Crops Research Branch, Agricultural Research Service, United States Department of Agriculture and University of Missouri, Columbia, Missouri: ***Anthocyanin synthesis in maize, the interaction of A_2 and Pr in leucoanthocyanin accumulation.***—In the aleurone tissue of the corn kernel, the *Pr-pr* factor pair controls the level of hydroxylation of anthocyanin pigment, purple *Pr* kernels containing glycosides of cyanidin (pentahydroxy), and red *pr* kernels containing glycosides of pelargonidin (tetrahydroxy). The differing hydroxyl group is that at the 3′ position on the B ring of the molecule. Aleurone tissue of colorless a_2 kernels contains a leucoanthocyanin which is converted to anthocyanin on heating a dilute acid-alcoholic extract of the tissue. The constitution of this anthocyanin is dependent upon *Pr* constitution, since extracts of a_2 *Pr* kernels give rise to cyanidin, while extracts of a_2 *pr* kernels give rise to pelargonidin. On the simplest assumption, it appears that the molecular difference controlled by *Pr-pr* is established rather early in the sequence of reactions leading to anthocyanin production.

CONGER, ALAN D., Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee: ***How oxygen causes an increase in chromosomal aberration yield.***—More chromosomal aberrations are produced by X-irradiation in the presence of oxygen than its absence. Observed aberrations are the result of two unseen events, primary breakage, and fusion of the breaks. Oxgyen could cause the increase in aberrations by increasing the primary breakage rate, decreasing the fusion rate, or by a combination of both. It is possible, in Tradescantia, to estimate the fusion of breaks and hence the degree to which fusion is affected by oxygen. The method is based on observation of the proportion of aberrations—of the isochromatid and chromatid interchange types—which are completely and incompletely fused. One can determine in these aberrations if the aberration is fused at both (proximal and distal) ends, only one end, or at neither end. Obviously, if oxygen caused more aberrations by decreasing the fusion of breaks, this would be manifested by a decrease in the proportion of completely fused aberrations, and conversely, by an increase in the proportion of incompletely fused aberrations. By such an analysis, the effect of oxygen on fusion of breaks was measured. Since both fusion and aberrations were measured, it was possible to solve for the numerical relation among the three related factors, breakage, fusion, and aberrations, both in the presence of oxygen and in its absence. It was found that: (1) primary breakage is increased by oxygen, equally for both chromatid and isochromatid breaks, (2) fusion of isochromatid breaks is the same with and without oxygen, i.e., for equal numbers of breaks equal numbers of aberrations result, and (3) fusion of chromatid breaks is about twice as great with oxygen as without, i.e., for equal numbers of breaks only half as many result in aberrations with oxygen as without. The measured effect of oxygen on breakage and fusion would satisfactorily account for the observed increases in aberration yield of all three types of aberrations, chromatid deletions, isochromatid deletions and chromatid interchanges. Thus it can be said that in Tradescantia oxygen causes an increase in X-ray-induced aberration yield by increasing the primary breakage rate.

CRAIG, DORIS L., J. S. KIRBY-SMITH, and J. N. DENT, Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee: ***Mitotic inhibition and chromosome breakage in salamanders of the genus Gyrinophilus.***—Mitotic inhibition and chromosome breakage by X-rays in regenerating tail tips of salamanders of the genus Gyrinophilus have been investigated. These studies have included work on the blind cave species *Gyrinophilus palleucus*. Regenerating tail tips of larvae were amputated and were treated for 6–8 hours with a 0.1% solution of colchicine prior to fixation with Sanfelice-White fluid. No colchicine was used on preparations for the mitotic inhibition study. Feulgen squashes were prepared in all cases. This procedure with colchicine yields preparations in which the chromosomes are comparable in size and clarity of structure to those of plant materials. Unambiguous chromosome

counts can be made with ease. The diploid number was found to be 28 for the 3 species examined (*G. palleucus*, *G. porphyriticus porphyriticus*, and *G. danielsi dunni*). The number of tail-tip cells in mitosis following 100r of X-rays (animals shielded) indicates that these cells are quite sensitive to radiation. The period of minimal mitotic activity extends from about 8 to 22 hours following treatment. X-rays produce the same types of aberrations in tail-tip chromosomes as those found in irradiated plant chromosomes. Chromatid and isochromatid aberrations and symmetric and asymmetric exchanges were observed at 48 hours following irradiation with 100 and 200r of X-rays.

CRARY, D. D., and P. B. SAWIN, Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine: *Some "nonspecific" (racial) and specific gene effects upon regional growth in the domestic rabbit.*—Adult enlarged growth areas in specific regions of the axial skeleton are known in two closely bred and selected races of rabbits (III, a New Zealand White and X, CASTLE's small sized race). These enlarged areas are already manifested in embryos of 20 and 21 days by the incidence and size of the ossification centers of the neural arches and centra of the vertebrae. At this age ossification is first appearing and progressing rapidly. Although there is some variation, the same basic pattern, consisting of three groups of advanced vertebral centers (cervical, thoracic and thoraco-lumbar) with relatively retarded groups separating them, is found in both races and in two sublines of one of them (III and IIIc). The pattern is varied significantly in precocity of first appearance and in magnitude of localized development (both longitudinally and laterally) in each group. Incidence of ossification centers is more advanced in race X of small adult body size than in III of large size. Precocity, however, is not a general size difference, since one subline of the same sized race (IIIc) shows similar precocity with significant localized differences measurable in time, IIIc being the more advanced. Similarly, the patterns of growth of these areas, both in length and width are quantitatively different and independent of each other. Like studies of the growth influences induced by scoliosis and dominant achondroplasia (*Da*) show similar localized influences in the axial skeleton and provide an approach to genetic analysis of growth forces responsible for a wide variety of sporadic morphological variations.

DELAMATER, E. D., E. J. MINSAVAGE and C. G. TOSTESON, University of Pennsylvania, Philadelphia, Pa.: *Synchronization of nuclear and cytoplasmic division in Bacillus megaterium.*—Synchrony of nuclear and cellular division in *Bacillus megaterium* was achieved by means of cold shock. Cell counts, culture density and cytological preparations were made at frequent intervals during the period of exposure to cold and subsequently during logarithmic growth at 34°C. The sequential phases of mitosis, consisting of prophase, prometaphase (unipolar metaphase), metaphase, anaphase, telophase and interphase, were followed statistically. The time required for each phase of mitosis has been analyzed.

DELAMATER, E. D., D. MCN. SCOTT and E. J. MINSAVAGE, University of Pennsylvania, Philadelphia, Pa.: *Correlation of nuclear and metabolic events during bacterial division.*—The time sequences of cytologically observable nuclear events and cytoplasmic division, and chemically determined increases in nucleic acids and acid soluble components have been correlated, and will be discussed.

DIRKS, V. A., J. G. ROSS and D. D. HARPSTEAD, South Dakota State College, College Station, South Dakota: *Colchicine-induced true breeding chimeral sectors in flax.*—The epicotyls of F_1 seedlings of a flax cross Crystal × B5128 were coated with 0.5% colchicine in lanolin as soon as the primary cotyledons opened. The treated epicotyls formed a typical C-tumor growth and subsequently new shoots arose from this growth. Seed from each branch was harvested separately and was later sown in separate rows. One F_1 plant of the cross Crystal × B5128 had branches differing in the color of seed produced. Three of these branches had brown seed (like B5128), two yellow seed (like Crystal) and one a mixture of yellow and brown seeds. The true breeding nature of the first five branches was established by observations made on three generations. Wilt readings indicated a striking difference in susceptibility of the progenies from different branches. Very little difference was found in general between the progenies of the brown seeded group and those of the yellow seeded group indicating that the original treated F_1 plant must have consisted of at least two chimeral sectors each breeding true.

The chromosome numbers of these strains were unchanged from the diploid. The origin of these true breeding chimeral sectors may be explained by reductional groupings of the chromosomes induced by colchicine treatment followed by restoration to the diploid number. A cell so formed could form a new growing point which would give rise to one of the branches of the flax plant.

DOLLINGER, E. J., Brookhaven National Laboratory, Upton, New York: *Analysis of a mutable system in maize.*—A mutable system occurred in a gamma radiation study. Mutation is controlled by a dominant factor *Ma* (mutation-activator). *Ma* will transpose to various locations in the complement. "Duplication" and movement of *Ma* may give rise to two independently assorting *Ma* factors. An individual plant possessing *Ma* and appropriate dominant factors when crossed to individual recessive testers for chromosomes 3, 5, 9, and 10 will result in mutation (chiefly recessive mosaic phenotypes) in 60 to 100% of the *Ma* endosperms. Expression of mosaic phenotypes is dependent on dosage of the locus studied; one dominant factor giving many mosaics, two dominant factors giving few mosaics since two simultaneous events are required for expression, etc. Analysis of dominant to recessive mosaics on chromosome 9 (*C-Wx*-centromere) reveals mutation $C > CWx > Wx$, plus some breakage fusion bridge cycles among others. Analysis of dominant to recessive mosaics on chromosome 5 (A_2-centromere-*Bt-Pr*) reveals mutation $A_2 > Pr > Bt$, plus mosaics involving two or three loci. Analysis of mutant sectors involving two or three loci indicate many may be accounted for by the induction of chromosome rings resulting from breakage at various points along chromosome 5. Analysis of early occurring mutation (large mosaic areas) indicate 50 to 80% may be accounted for by induction of chromosome rings. It appears possible that every chromosome may have a number of sites where *Ma* will induce breakage; breakage being more prevalant near chromosome ends. (Work carried out at Brookhaven National Laboratory under the auspices of the U. S. Atomic Energy Commission.)

DUVICK, D. N., Pioneer Hi-Bred Corn Company, Johnston, Iowa: *Allelism and comparative genetics of fertility restoration in cytoplasmically pollen sterile maize.*—Six lines of maize: KY21, K55 (Agricultural Experiment Station inbreds), WG3, BH2, F5DD1 (Pioneer Hi-Bred Corn Co. inbreds), and Zapalote Chico (Mexican open pollinated variety) restore pollen fertility to cytoplasmically pollen sterile ("Texas" source) WF9. Loci of all or nearly all of the fertility restorer (*FR*) genes of each of the first 5 lines were shown to be allelic, but most or all of the *FR* loci of the last line, Zapalote Chico, were non-allelic with loci of the first group. When WF9 was crossed and backcrossed to each of the above 5 *FR* inbreds (in "Texas" cytoplasm) the proportions of sterile to fertile plants in each backcross population indicated that 2 dominant complementary genes were required for complete fertility restoration. When 3 different cytoplasmically pollen sterile inbreds ("Texas" cytoplasm): WF9, K4 and C106 were compared as recessive testers in combination with BH2 and with KY21, backcross data indicated that: (1) WF9, K4 and C106 differ with respect to the number, and possibly the degree of recessiveness, of recessive loci concerned with cytoplasmic pollen sterility; (2) BH2 and KY21, although appearing to have allelic and approximately equal sets of dominant complementary genes for fertility restoration with respect to WF9 and to K4, are shown in backcrosses to C106 to have unequal powers of restoration of pollen fertility. This could be due to the presence of one or more genes in BH2 which are more strongly dominant for fertility restoration than are their alleles in KY21. Comparisons of segregating backcross populations grown in Florida (winter) and Iowa (summer) suggest that for most or all of the 5 *FR* inbreds at least one major dominant gene is required for *FR* in any location. If the environment is sufficiently conducive to pollen fertility, only this gene is required. If the environment is more severe (Iowa summer) one or more dominant complementary genes (depending on the lines concerned) are needed in addition, for full pollen fertility.

EDINGTON, CHARLES W., Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee: *The composition of sex-linked recessive lethals induced in Drosophila by radiations of different ion density.*—These investigations were undertaken in an effort to gain further information concerning the origin of sex-linked recessive lethals. Oregon-R males were exposed to fast neutrons in the Oak Ridge 86-inch cyclotron or to Co^{60} gamma-rays and the frequency of induced sex-linked recessive lethals determined. When the regression for the lethal frequency against dose is plotted for neutrons

and for gamma-rays, the relative biological effectiveness (RBE) of neutrons to gamma rays is about 2, whereas the RBE for neutrons to X-rays (by the regression of TIMOFÉEFF-RESSOVSKY 1939) is only 1.2. The frequency of recessive lethals induced by fast neutrons in a ring-X, X^{cl}, and a rod-X, *In(1)EN* (resulted from an opening out of the X^{cl} chromosome) was also determined at four doses. The slopes of the regressions indicate that the frequency of lethals induced in the ring chromosome is significantly lower than the frequency induced in the rod chromosome. Since the RBE for dominant lethals and translocations has been reported to be quite high (4–7), it would seem valid from the results reported here to conclude that an appreciable number of recessive lethals arise as the result of primary breakage and are independent of the eucentric manner in which the breaks rejoin.

ELLIS, J. F., P. T. IVES and R. EARLE, JR., Amherst College, Amherst, Mass.: *The combined effects of gamma radiation and ether on the frequency of dominant lethals in Drosophila melanogaster.*—Tests were made of the effects of 300r gamma from Co^{60} at 10r/minute, combined with various numbers of etherizations before and after radiation, on the frequency of egg-lethal mutations in the mature sperm of young inbred Samarkand males which were mated to sibs in single pairs. Each pair was checked for virginity and fertility before its frequency of non-hatching eggs was included in the data. Controls for all series together averaged near 4% lethals and showed no apparent ether effect. In series testing 300r with 1 etherization 4 hours before and a second 4 hours after radiation the lethal frequency was near 8%. With 5 to 10 etherizations spaced hourly before or after radiation, or both, the lethal frequency dropped to near 5%. Four groups of tests gave similar results and in 3 of them the egg counts were large enough to make each group independently significant at the 2% level or lower. One similar group of tests of 3000r at 100r/minute showed differences in the same direction and of similar absolute (not proportionate) amount, significant at the 5% level. One possibility is that etherization may create an oxygen deficit under which conditions the frequency of recovered radiation-induced chromosome breakage is known to be reduced.

EVERETT, H. L., and P. J. LOESCH, Cornell University, Ithaca, New York: *Studies on the restoration of pollen production in cytoplasmic sterile maize.*—An aberrant cytoplasm and/or cytoplasmic particles have been postulated as the controlling factor for pollen sterility in specific stocks of *Zea mays* L. This male sterile condition may be partially or completely overcome in at least three ways. External environment, dominant gene action, and systemically active chemical sprays will be considered as agents of restoration.

FABERGÉ, A. C., University of Missouri, Columbia, Missouri: *Types of chromosome aberrations induced by ethylene oxide in maize.* Ethylene oxide has been shown to produce marker losses in maize endosperm, by SMITH and SRB (1952, Maize News Letter 26:28). KOLMARK and GILES (1953, Genetics 38:674) found that the related diepoxybutane shows great specificity in Neurospora, giving many more adenineless than inositolless reversions as compared with radiation. Pollen carrying *I Sh Bz Wx* was treated, and used to pollinate plants carrying *C sh bz wx*, an arrangement which allows the identification of a range of aberrations by the endosperm phenotype. Treatment consisted of tumbling small portions of pollen in a rotating glass tube 1 cm in diameter, through which a mixture of 1 part ethylene oxide to 20 parts air by volume flowed at 50 cc per minute. A 4 minute exposure reduced the set drastically, and a 2 minute exposure slightly. Roughly 1% of kernels had identifiable aberrations in the marked short arm of chromosome 9, about $\frac{1}{10}$ of the rate that could be achieved at the same sterility level by radiation. Within the rather large uncertainties due to small sample size and difficulties of comparing different equivalent doses, the effects of ethylene oxide seem quite similar to those of X-rays, the same kinds of aberration being produced in about the same proportions. (Work supported by a research grant from the National Cancer Institute, National Institutes of Health, U. S. Public Health Service.)

FARNSWORTH, M. W., University of Buffalo, Buffalo, N. Y.: *Effects of some homozygous Minutes on the development of Drosophila melanogaster.*—The effects on embryonic development of homozygous *M(1)o*, *M(2)1*, *M(2)1²*, *M(3)w*, *M(3)B*, *M(3)B²*, *M(3)124*, and nullo-4 are being investigated. Of

these, *M(1)o* and *M(2)l*[3] are generally lethal in the first larval instar, while *M(2)1*, *M(3)w*, *M(3)B*, and *M(3)124* die in the egg stage. *M(1)o* homozygotes usually hatch from the egg within 22–24 hours after the initiation of development although some larvae, apparently unable to escape, have been observed moving inside the vitelline membrane 12 hours after normal hatching time. In general, homozygous *M(1)o* larvae exhibit feeble feeding actions and often move only when stimulated. Some individuals, however, survive up to 5 days after hatching although they show no appreciable gain in size or degree of activity during this period. Preliminary examination of sectioned *M(2)1* homozygotes reveals a pattern of abnormalities which closely follows that displayed by *M(4)*. Lethal individuals can be identified with certainty only after 12 hours of development. As in the case of *M(4)*, abnormalities involve the gut and nervous system while yolk utilization is impaired. The evidence so far indicates that the homozygous *M(2)1* deletion results in a generalized disturbance in correlations and metabolism rather than an effect exerted specifically on a single organ or system. (Supported by a grant-in-aid from the American Cancer Society upon recommendation of the Committee on Growth of the National Research Council.)

FINGER, I., University of Pennsylvania, Philadelphia, Pa.: *The inheritance of ciliary antigens in Paramecium aurelia, variety 2.*—Cultures of *Paramecium aurelia* when injected into rabbits elicit antibody formation against animals of the injected type. Serum prepared against clones of Paramecium pure for a specific immobilization antigen will, in proper concentration, immobilize those organisms having the same ciliary antigen as the paramecia injected and will not react with other paramecia. Studies with variety 2 of *P. aurelia* have demonstrated that the inheritance of these antigens in this variety follows a pattern similar to that already shown for varieties 1 and 4: single genes control whether an antigen is manifested and the kind of antigen synthesized. The cytoplasm determines which antigen of the several possible antigens a paramecium can produce is actually manifested at a particular time. In animals presumed homozygous for all loci the appearance of a serotype ordinarily manifested only by a heterozygote has been shown most likely to be due to a chromosomal abnormality. Serological experiments have demonstrated that a serotype controlled by a single locus possesses at least two antigenic groups, at least one of which is unique for a particular allele at the locus. The antigenic groups are situated on a single molecule.

FORSTHOEFEL, PAULINUS F., University of Detroit, Detroit, Mich.: *The interaction of the luxoid and luxate genes in the offspring of C57BL mice heterozygous for both genes.*—On the C57BL genetic background, the luxoid and luxate genes when each is present separately in homozygous condition produce the characteristic effects on the skeleton described elsewhere. When each is present in heterozygous condition singly, they produce polydactyly of the hind limbs. When both are present together in heterozygous condition, they interact to produce a type of hemimelia often distinguishable from that produced by either the luxate or luxoid genes when in homozygous condition. Using as criteria these characteristic effects, offspring of parents heterozygous for both genes were classified into eight phenotypes, the animals singly heterozygous for luxate or luxoid being classified together since phenotypically indistinguishable. The numbers observed for each class agreed well with the numbers expected. The *+lu lxlx* animals showed complete absence of tibia, a straight fibula, four toes on the hind feet, and in two cases a fragmented femur. The *lulu +lx* animals showed polydactylous forefeet, replacement of tibia and fibula by a variously shaped small bone, four toes on the hind feet, and in one case reduction of the femur to a fragment. The *lulu lxlx* animals showed polydactylous forefeet, replacement of femur, tibia, fibula by a single short rod of cartilage, and three toes on the hind feet. It may be concluded from these observations that the luxoid and luxate genes interact additively in their effects on the hind limbs and that they are not lethal in double homozygous condition. (Supported by a grant from the National Science Foundation.)

FUNG, SUI-TONG CHAN, and JOHN W. GOWEN, Iowa State College, Ames, Iowa: *Pigment inducing potentialities of testes, ovaries and hermaphroditic (Hr) gonads.*—Wild type testes and vasa deferentia of adult males in *Drosophila melanogaster* are yellow in color. The ovaries and the lateral oviducts in the females are colorless. In our specialized intersex Hr, tissues having yellow pigments characteristic of the testicular epithelial sheaths are generally present in small areas or minute spots closely

associated with the gonads and genital systems. Transplantation of wild type male genital imaginal discs into males and females of "colorless"(*w*) genotypes showed that the vasa deferentia remained colorless. When transplanted into normal male, female or Hr hosts, one or both sides of the vasa are capable of developing yellow pigments. Potentiality of the Hr gonads was tested by comparing the Hr-vas relationship with those of normal males and females. Implantation of normal testis, ovary of Hr gonad into males of "colorless"(*w*) constitution showed that Hr gonads behave like normal ovaries in initiating yellow pigmentation. Normal testis induced pigment formation in the "colorless" host gonad and yellow pigments appearing as thin coating extended to the vasa deferentia. When the normal ovary was transplanted into *w*-males, the vasa of the "colorless" host became yellow but no yellow pigment was found in either the host or donor gonad. Similar results were obtained when the Hr gonad was introduced into the "colorless" male host. In all cases, contact between hosts and implants seems necessary. Reciprocal transplantation of "colorless" gonads into hosts of normal and Hr genotypes demonstrated an exchange of pigment forming substance or cells between hosts and implants. Testis of "colorless" constitution possessed yellow and colorless areas in a mosaic pattern when transplanted into normal male host. When transplanted into normal females, a very faint yellow pigmentation appeared in the "colorless" implanted testis. In the Hr host, the "colorless" implant also possessed mosaic yellow and colorless areas. It seems evident that not only testes but ovaries and Hr gonads are capable of inducing pigment formation in the vasa as well as gonads of "colorless" constitution. The quantity of pigment present in the ovaries and Hr gonads, as determined by the amount deposited in the gonads and vasa, is comparatively less than that found in normal testes. (This work has received assistance from contract No. AT(11-1)107 from the Atomic Energy Commission.)

GARBER, E. D., The University of Chicago, Chicago, Ill.: *The orientation of interchange complexes and quadrivalents at metaphase I in Sorghum purpureo-sericeum.*—*Sorghum purpureo-sericeum* has a somatic chromosome number of 10. The orientation of ring interchange complexes in this species is apparently not directed since the open and zigzag configurations at MI occur with approximately equal frequencies. The orientation of both ring and chain quadrivalents in an induced autotetraploid of this species is likewise apparently not directed since the open and zigzag (alternate) configurations occur with approximately equal frequencies at MI. These observations support a previously proposed hypothesis that if a species does not display a directed orientation of the chromosomes in an interchange complex at MI, the quadrivalents of an induced autotetraploid of this species will likewise not display a directed orientation at the same stage. (Aided by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of The University of Chicago.)

GODFREY, GEORGE F., Oklahoma A. & M. College, Stillwater, Okla.: *Genetic variation and covariation in broiler body weight and breast width.*—Heritability estimates of 9-week body weight and breast angle, and genetic, environmental and phenotypic correlations between these traits were calculated from variance and/or covariance components, and by intrasire regression methods, for males and females of Silver Oklabars and New Hampshires. The mating system was one of restricted random mating. Silver Oklabar population size was 14 sire families, 110 dam families, 343 male progeny and 335 female progeny. Similar figures for the New Hampshire population were 12, 120, 345 and 328 respectively. Heritability estimates of 9-week body weight for the Silver Oklabars and New Hampshires averaged 0.51 and 0.43; estimates of 9-week breast angle averaged 0.44 and 0.48 respectively. The genetic correlation between these two traits averaged 0.47 for Silver Oklabars and 0.57 for New Hampshires. Phenotypic correlations averaged about 0.32 for both breeds. Environmental correlations were 0.42 for Silver Oklabars and 0.22 for New Hampshires. Selection for 9-week body weight should result in a concurrent improvement in breast angle in these two populations.

GRAF, GERARD E., SUI-TONG CHAN FUNG and JOHN W. GOWEN. Iowa State College, Ames, Iowa: *Paper chromatographic analysis of hermaphrodite gonads in Drosophila melanogaster.*—The dominant third chromosome gene (Hr-Gowen) in *Drosophila melanogaster* affects diploid females, producing sterile individuals with duplication of male and female secondary reproductive systems.

The presence of yellow pigmented tissue associated with the reproductive ducts of these Hr females is suggestive of the yellow pigment found in the external epithelial sheath cells of normal testes. The present work demonstrates that these yellow pigments are in fact identical.

Using essentially the chromatographic methods first described by HADORN and MITCHELL, the yellow pigment was isolated from testes of wild type males and from the yellow organs of the hermaphrodites. The two pigments were chromatographically identical using propanol-1% NH_3 (2:1) and butanol-acetic acid-water (4:1:5) as developers. The pigments were also chromatographically identical with the yellow pigment in wild type eyes. Wild type ovaries did not show this pigment. Furthermore the yellow pigment of both wild type testes and the yellow organs of the hermaphrodites was broken down by ultraviolet light to a blue fluorescing substance which was chromatographically identical with 2-amino-4-hydroxypteridine-6 carboxylic acid. Isoxanthopterin was also found in the wild type testes and the yellow organs of the hermaphrodites but not in wild type ovaries. A blue fluorescent compound was found in wild type ovaries and hermaphrodites but not in wild type testes. Hence the hermaphroditic reproductive tract appeared chromatographically to have the characteristics of both male and female wild types. (This work has received assistance from contract No. AT(11-1)107 from the Atomic Energy Commission.)

HERSKOWITZ, I. H., Indiana University, Bloomington, Ind.: *The incidence of chromosomal rearrangements and recessive lethal mutations following treatment of mature Drosophila sperm with 2:4:6-tri (ethyleneimino)-1:3:5-triazine.*—Following the proof that under certain conditions translocations induced by a N-mustard solution can be as frequent as those produced by X-rays, at doses of both mutagens yielding equal frequencies of recessive lethal mutations (see SCHALET, these Records), experiments were performed with the partial purpose of testing whether 2:4:6-tri(ethyleneimino)-1:3:5-triazine ("triazine") behaved in a similar manner. After postcopulatory vaginal douche treatments of inseminated females, the following types of mutations in sperm-contributed chromosomes were detected employing MULLER's (D.I.S. 28) standard "multi" technique: partial or complete loss of the sex chromosomes, X-chromosome recessive lethal mutation, and translocation. In one experiment females were treated with approximately 1% triazine solution and transferred to new food-containing vials 4 times at 3-day intervals to furnish 5 successive broods. Mutations detected in offspring from the first two broods, the third brood, and the last two broods were, respectively, for sex-chromosomal losses, 0.4% (3 mutations/747 tests), 0.6% (5/854), and 0.4% (3/733); for recessive lethals, 6.8% (44/648), 6.8% (53/784), and 6.9% (43/623); for translocations, 0.4% (2/532), 2.4% (14/573), and 5.7% (31/544). Although the frequencies of sex-chromosomal losses and of recessive lethals were not significantly changed in successive broods, the incidence of translocations was significantly higher in the sperm held longer before fertilization. This extends to triazine SCHALET's observations for N-mustard. There were 68 "2-3", 14 "Y-2", and 11 "Y-3" translocations detected in various experiments with triazine, a distribution similar to that obtained with X-rays (MULLER, HERSKOWITZ and OSTER unpublished), but different ($P < .01$) from that with N-mustard (SCHALET), for the same sperm chromosomes treated in inseminated females. (This work has been supported by a grant received for work of H. J. MULLER and associates from the American Cancer Society, on recommendation of the Committee on Growth of the National Research Council.)

HERSKOWITZ, I. H., and S. ABRAHAMSON, Indiana University, Bloomington, Ind.: *The dependence of chromosomal rearrangements in oocytes of D. melanogaster upon X-ray delivery rate.*—That chromosome rearrangement frequency varies with X-ray delivery rate has been definitely established for plant but not for animal cells. The present establishment of this relationship in Drosophila became feasible following the demonstration that many chromosomal rearrangements of the half-translocation type are produced by X-rays in oocytes. Virgin females carrying attached-X ("snoc") and partial-Y ($sc^{v_1}.Y^S$) chromosomes were aged at least 3 days, irradiated, and mated to males carrying an X (*y* sc^{S_1} *B In49 v*) and a Y chromosome. F_1 flies, from eggs oviposited within 4 days after treatment, were scored for detachments of the attached-X's (detected if one arm was lost), in three pairs of experiments: 2000r were delivered (A) either continuously at 600r/min. (yielding 5.4% detachments) or during 2 equal periods 8 hr. apart at 30r/min. (1.5% detachments); (B) at 600r/

min. either continuously (1.9% detachments) or in 4 equal periods at 8-hr. intervals (0.9%); (C) 1000r were delivered uninterruptedly either at 1100r/min. (1.6%) or at 30r/min. (0.7%). For each paired comparison $P < .005$. In another experiment a total of 2200r were delivered at 1100r/min. during 8 equal periods with 0, ¼, ½, 1, 4, 16 or 32 min. between successive irradiations. Detachment percentages were similar when the periods between irradiations were 4 min. or less, averaging $7.1 \pm 0.35\%$, decreased from this to $5.3 \pm 0.64\%$ ($P = .025$) with the 16-min. interval, and to $3.4 \pm 0.41\%$ ($P < .0001$) with the 32-min. interval. These results prove that breaks in oocytes can join in new arrangements before fertilization, unlike breaks in mature sperm, but seldom join before 4 min. have elapsed since their production. (This work has been supported by a grant to Dr. H. J. MULLER and associates from the U. S. Atomic Energy Commission (Contract AT (11-1)-195).)

HILLMAN, RALPH, Yale University, New Haven, Conn.: ***Neurological effects of a mutation affecting the head of Drosophila melanogaster.***—Genetic reduction of the eye of Drosophila results in reduced optic and antennal glomeruli in the adult brain (POWER 1943, 1946). A mutation which has appeared in certain Notch stocks results in reduction of the eye, frequently accompanied by duplication of the antenna and ocelli. The expression of this mutation has been limited to females heterozygous for Notch, and increased penetrance is dependent upon a low temperature effect. The reduction in eye size is variable and may be accompanied by a reduction in the cuticle normally derived from the eye-imaginal-disc. The duplications of antennae and ocelli may be either partial or complete, and the expression of the mutant may be either unilateral or bilateral in nature. Histological sections of the heads of such flies show that the duplicated organs are innervated by branches of the antennal and ocellar nerves. Correlated with eye reduction is a reduction in the size of the optic glomeruli. The brain itself often appears asymmetrical with a medial displacement of the protocerebral lobe attended by a disruption in the neural tracts. These neural effects are being studied with regard to the question of the origin of the sensory nerves in Drosophila.

HINTON, CLAUDE W., Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee: ***Analysis of an unstable ring-X chromosome and its derivatives in Drosophila melanogaster.***—Instability of the w^{vc} ring-X chromosome of *D. melanogaster* leads to the production of gynandromorphs, XO males, dominant lethals, and (rarely) small unstable rings consisting chiefly of the w^{vc} centric and heterochromatic regions. Formation of anaphase bridge configurations by the w^{vc} chromosome accounts for these results; thus gynandromorphs and XO males register bridge loss whereas dominant lethals and small rings arise by inclusion of bridge breakage products in the cleavage nuclei. Maintenance of instability by small rings derived from w^{vc} shows that control of instability is probably a property of some heterochromatic element of the w^{vc} chromosome. Sister-strand crossing over has been proposed as the basis for anaphase bridge formation by the ring chromosomes of maize. To test whether this mechanism explains w^{vc} instability, metacentric rod chromosomes were obtained as crossover products having the w^{vc} centric and heterochromatic regions intact; sister-strand exchanges in rod chromosomes should not produce anaphase bridges. Comparison of w^{vc} metacentric behavior with that of w^{vc} rings generated from the metacentrics by crossing over shows that both chromosomes are unstable to about the same extent although the metacentric produces very few gynandromorphs. This difference in the manifestation of w^{vc} instability by the metacentric and the ring might be related to a difference in the type of bridge formed by the two chromosomes; both types of bridges may be formed by sister-strand union.

HOLLANDER, W. F., and J. W. GOWEN, Iowa State College, Ames, Iowa: ***Estimation of the induction rate for dominant lethal effects in mouse sperms by X-rays.***—Probably because of its larger size, the mouse sperm is several times as sensitive to ionizing radiation as that of Drosophila. Impregnations from males given 1500 roentgens, 250 pkv 30 ma 0.25 cu + 1 al filter X-rays, rarely produce any viable embryos. The present study involved over 800 pregnancies examined by dissection, including over 150 controls. The range between 0 and 1200r was tested by 100r intervals. To avoid complication of mortality with recessive lethals, all matings were crosses, and to insure maternal vigor females less than 9 months old were used. Nevertheless, embryonic mortality in control matings was about 10%, chiefly occurring shortly after implantation. Implantation sites remained

evident until parturition provided at least one fetus is alive. Dosages through 600r showed a rise in mortality to over 50%, chiefly at stages soon after implantation. The average number of implantations showed no marked decline up to 600r, but a decline was obvious at higher doses; at 1200r this amounted to nearly 50%. Complete mortality within a uterus became significant by 800r and timed matings, and dissection on the tenth day of gestation indicated that at 1200r about a third of all pregnancies were in this class. With correction for probable loss before implantation, the mortality at 1200r seemed to be 90%. The proportions of surviving embryos are roughly described by an exponential. (This work has received assistance from contract No. AT(11-1)107 from the Atomic Energy Commission.)

HOUSE, VERL L., Radford College, Radford, Virginia: *The influence of temperature on gene expression at the cubitus interruptus locus in Drosophila melanogaster.*—The fourth vein interruption effects of alleles at the cubitus interruptus locus vary differentially with temperature changes. Cubitus interruptus (*ci*), a recessive, exhibits a linear decrease in interruption with increase in temperature. Cubitus-interruptus-dominant (ci^D), a homozygous lethal dominant, shows a linear increase in L_4 interruption with increasing temperature. Cubitus-interruptus-Wallace (ci^W), a partial dominant, exhibits a more complex temperature response, showing relatively little change in L_4 interruption between 14°C and 22°C, a sharp increase in L_4 interruption between 22°C and 28°C, and a decrease in interruption between 28°C and 30°C. Other differential effects of these alleles, either in response to temperature changes or at a given temperature, include interruption of L_4 and L_5 proximal to the posterior cross vein, distal interruption of L_2 and L_5, and scalloping of the posterior wing margin. The latter effect is accentuated in *ci* by decreasing temperature and dosage, while ci^D shows increased scalloping with increase in temperature. A previously employed threshold interpretation of the penetrance and expressivity of the *ci* allele is supported and extended to ci^W as a result of the high correlations obtained between penetrance and expressivity and between penetrance and bilateral expression for the two alleles over the temperature range employed. (Work aided by a Grant-in-Aid of Research of The Society of The Sigma XI.)

HUESTIS, R. R., and RUTH S. ANDERSON, University of Oregon, Eugene, Oregon: *Inherited spherocytosis in Peromyscus.*—Continued study has been made of an inherited syndrome in Peromyscus characterized by neonatal jaundice, splenomegaly, reticulocytosis and spherocytosis. The opinion that a single recessive gene is a main etiological factor has been confirmed by the results of reciprocal test crosses and pure line matings. Reticulocyte counts of blood smears of adult mice of the three genotypes; affected, heterozygous and normal, have averaged 15, 3 and 2 percent respectively. The small difference between heterozygous and normal mice is of doubtful significance. Fragility tests of the erythrocytes of the same three genotypes show beginning hemolysis in affected mice at about .74 and in heterozygous and normal mice at about .50 percent saline. The intensity of the neonatal jaundice among affected sibs is variable and as the incidence of intense jaundice is greater among the offspring of previously intensely jaundiced male parents this variability appears to be genetic. The weight of hypertrophied spleens may be from 3 to 10 times the weight of normal spleens in mice of comparable size and age. The genetic implications of this have not been tested. (This work has been supported by grants from the Graduate School of the University of Oregon and from the National Institute of Arthritis and Metabolic Diseases, U. S. P. H. Service.)

IVES, P. T., R. D. BAUGHMAN and W. G. GAMBLE, Amherst College, Amherst, Mass.: *A twelve point mutation rate curve for gamma radiation in Drosophila melanogaster.*—Using young males of inbred Oregon-R and Basc females the sex-linked mutation rate in mature sperm was tested in controls and at 11 Co^{60} radiation dosages ranging from 50r to 12,000r, chiefly at 1500r intervals, with intensities of 10r/minute for low doses and 100r/minute for doses of 1500r and higher. Matings of single males were used in testing doses of 50r to 9000r and the number of F_1 tests/radiated male was regulated and recorded in families. The family data show a good fit to a Poisson series. In tests of 3000r and higher the F_1 females were mated to stock or control Basc males. (Tests with sib males showed a significantly lower mutation rate and a linearly increased frequency of sterile tests.) This method reduced sterility markedly and under these conditions, when allowance was made for a

Poisson accumulation, the mutation rate increased apparently more than linearly with dosage, from .12% in 10,521 control tests and .71% in 8402 300r tests to 34.6% in 133 10,500r tests and 36.1% in 108 12,000r tests. More data are needed to reduce the fiducial limits of points above 4500r. Of several explanations for the possibly greater than linear increase in mutation rate the authors favor the interpretation that two "weak" mutations unnoticed when acting singly are sometimes strongly mutant when present together in a chromosome.

IVES, P. T., R. S. HEILMAN and H. H. PLOUGH, Amherst College, Amherst, Mass.: *The LD_{50} for gamma radiation in Drosophila melanogaster.*—Young flies from inbred Oregon-R were subjected in groups of 100 males and 100 females to Co^{60} gamma radiation in doses ranging from 25,000r to 250,000r at 225r/minute. The flies were kept 10/vial at 24 ± 1°C and were shaken to fresh food every 2 days. Survival and mortality curves were constructed. Males proved relatively more sensitive to radiation than did females. In terms of the median, mode and mean mortality for controls and 4 dosages (25,000r to 100,000r) the interpolated LD_{50} is near 40,000 for males and near 60,000r for females. After receiving 100,000r males live for about two weeks and females for nearly four weeks before "radiation sickness" (loss of coordination) begins, leading to death within a few days. After receiving 150,000r flies walked normally, none could fly, only a few females could feed and lived longer than 1 day, dying within a week. After 200,000r or 250,000r flies showed only small uncoordinated movements (similar to those in mild anesthesia) larger in the lower dose and more in females than in males, followed by death within a few hours. Preliminary tests suggest that less than 10,000r prevents further germ cell production, but occasional mature sperm produced fertile F_1 after receiving as much as 16,000r.

JAMES, ALLEN P., and C. E. CHAPLIN, Atomic Energy of Canada Ltd., Chalk River, Ontario: *The effects of recessive lethal mutations in the heterozygous state in yeast.*—A technique for studying a representative sample of radiation-induced recessive lethal mutations in yeast populations is described. The ability of heterozygotes to compete with lethal-free cells is observed. A diploid culture of *S. cerevisiae* is irradiated and maintained in the vegetative phase. At intervals, the population is assayed for proportion of cells that contain recessive lethals. The identification of such cells is based on repeated failure to obtain more than two viable spores per ascus in derived lines. In a preliminary test, approximately 1.6×10^7 independent lethals were induced by ultraviolet among 10^8 cells. An initial decline in number of heterozygotes suggested that the average effect was deleterious. None of the heterozygotes had the competitive advantage displayed by two lethal-free but abnormally-sporulating cell types that arose in the population.

JENKINS, J. A., and G. MACKINNEY, University of California, Berkeley, Calif.: *Carotenoids of the Apricot Tomato.*—The phenotype of homozygous apricot, a new recessive mutant, differs from the common red varieties in having a low lycopene content (2 to 5 micrograms per gram of fresh fruit). In this respect it resembles the yellow homozygote. However, apricot differs from yellow in having a β-carotene content characteristic of red (6–10 μg/g).—The double recessive apricot-tangerine is very similar to the yellow-tangerine double recessive: the carotenoids of the lycopene set are decreased in amount and the main constituent is prolycopene, the characteristic carotenoid of tangerine. The two double recessives are essentially the same in their content of ζ-carotene, β-carotene and phytofluene. On the other hand, in the yellow-apricot double recessive, the suppressing action of the two genes is increased nonadditively. We did not detect any lycopene, and the content of β-carotene is lower than in the yellow. Each of the recessive genes, yellow, tangerine and apricot, alters the amounts of several carotenoids and in combination interact in a complex manner.

JOACHIM, GERTRUD S., University of Minnesota, St. Paul, Minn.: *Studies on the inheritance of "reversed germ" in corn.*—This character originated in an inbred line of field corn. The germinal face is turned toward the butt of the ear whereas normally it is turned toward the other end of the ear. The frequency of reversed germ kernels in this line varies from 5–65%, the majority being between 15 and 35%. The cross of the reversed germ ♀ × one normal line as ♂ gives a similar percent of reversed germ kernels on the crossed ear. The ear from the reciprocal cross is normal.

The ears on the F_1 plants have only normal kernels, indicating that the character is recessive. Based on the ears on the F_2 plants, there are about 9 with only normal kernels: 7 with various percentages of reversed germ kernels. The ratio from the F_1 backcrossed to reversed germ fits a 1:3 ratio; also indicating two recessive factors for reversed germ. The F_1 of another normal line ♀ × reversed germ as ♂ gives a low percentage of reversed germ on the crossed ear, ranging from 3.5% to 8%. The behavior of the ears on the F_2 and backcross plants of this group can be explained by an additional factor for reversed germ which is dominant. A second reversed germ line in field corn was furnished by DR. F. S. WARREN, Department of Agriculture, Central Experimental Farm, Ottawa, Canada. This line has a high percentage of reversed germ, from 60 to 90%. The ears on F_1 plants from the cross between this line and the Minnesota line have normal kernels, indicating that different factors are involved. Since these studies were started, two different lines with reversed germ have been found by those working with the corn genetics stocks at Minnesota.

KALTER, H., McGill University, Montreal, Quebec, Canada: *The influence of maternal weight and age on the cleft palate-inducing properties of cortisone in mice.*—(C57BL/6Jax ♀ × A/Jax ♂)F_1 (=BA) ♀ ♀ backcrossed to A/Jax ♂ ♂ and given 2.5 mg cortisone acetate daily for 4 successive days beginning on day 10, 11, or 12 post-conception, bore offspring with a 20% mean frequency of isolated cleft palate. The abnormality rate decreased with parity, from 31.5% in 1st to 5.4% in 7th–9th pregnancies (Sci. 118: 625, 1953). In a more critical experiment, 40 primiparous BA ♀ ♀, whose conception weights and parturition ages ranged from 19.8 to 30.8 grams and 63 to 165 days, respectively, were treated as described above, this time beginning on the 11th post-conception day only. The mice bore 315 examinable offspring, of which 171 (54.3%) were affected. The offspring showed, as did those in the previous experiment, a decreasing cleft palate incidence, with increasing maternal age and weight (80% in offspring of youngest, lightest ♀ ♀, 35% in those of oldest, heaviest ones). The difference in mean frequency of abnormal offspring between the 20 lightest ♀ ♀ (67.8%) and the 20 heaviest ones (43.3%) is statistically significant ($\chi^2 = 17.9$, $P = .0001$). With parity held constant, therefore, it is seen that heavier, older mothers are more resistant than lighter, younger ones to the cleft palate-inducing properties of cortisone. The correlations between weight (1), cleft palate frequency (2), and age (3), are: $r_{12} = -.639$, $P \ll .001$, $r_{13} = .793$, $P < .0001$, and $r_{23} = -.557$, $P < .001$. The partial correlation value, $r_{12\cdot3} = -.389$, $P = .01$–$.02$, indicates a significant correlation between weight and cleft palate frequency even when age effects are discounted. (Supported by a Postdoctoral Fellowship from the National Cancer Institute, United States Public Health Service.)

KAPLAN, W. D., and S. R. PELC, Medical Research Institute, City of Hope, Duarte, California, and The Radiotherapeutic Research Laboratory, Hammersmith Hospital, London, England: *Autoradiographic studies of Drosophila gonads following the feeding of C^{14} labelled formaldehyde.*—It has been shown that germ cells of male *Drosophila melanogaster* larvae exhibit varying rates of mutation following treatment with formaldehyde. The variability is a reflection of the age, at the time of treatment, of the germ cells. The pattern of sensitivity, however, is a constant one. In order to determine the basis for the pattern that has been observed, labelled formaldehyde was fed to male and female larvae at 24, 48, 72 and 96 hours of age and to adult males and females. Within the limits permitted by the technique it may be concluded that the sensitivity pattern is a reflection not of a differential penetration on the part of the formaldehyde into germ cells of different ages, but of a differential response to the presence of formaldehyde. A uniform concentration of formaldehyde was present throughout the gonads of all the stages examined. Female larval gonads, which remain refractory to the mutagenic action of formaldehyde, gave as marked an autoradiograph as male gonads of comparable age. Within the gonads of male and female adults that have been fed formaldehyde there was marked activity indicating the presence of the labelled carbon atom although it is known that mutations may not be induced in either male or female adults by the feeding of formaldehyde food.

KIMBALL, E., Clinton Experimental Farm, Clinton, Conn.: *Quail plumage pattern in the fowl.*—Phenotype in the Belgian Quail Bantam accords with modified columbian primary plumage pattern, more especially as observed in columbian × extended black hybrids (*E e*). Experiments demon-

strate, however, that the Quail Bantam is homozygous for a primary pattern gene, and does not carry heterozygous (*E e*). Further analysis shows black feather pigment in the Quail is *B*, as in extended black (*E*); whereas, the primary restrictor is *R'*, as in columbian (*e*). Test matings, and phenotypes of F_1, F_2, and backcross generations, provide evidence of *B* and *R'* linkage in the Quail; and that primary pattern genotype in Quail is ([*B R'*] [*B R'*]). The symbol $E^{R'}$ is proposed for the linkage [*B R'*]. Since prior studies show wild type (e^+) to be [*b R*], columbian (*e*) is [*b' R'*], extended black (*E*) is [*B r*], and birchen (E^R) is [*B R*], Quail ($E^{R'}$) adds another combination [*B R'*] to the series of gene-clusters affecting primary plumage pattern in Gallus. Cumulative evidence suggests primary pattern genes in the fowl are not single chromosomal points with pleiotropic potential, but rather, clusters of independent points subject to crossover and point mutation. An unusual self-black (extended black) form, demonstrated to carry *b*-black, instead of *B*-black, and therefore [*b r*] in constitution, has also been isolated. Thus, six of nine gene-combinations, theoretically possible, have been demonstrated. Incomplete evidence also suggests that Sebright Bantam carries ($E^{R'}$ $E^{R'}$) primary plumage pattern genotype, and not (*e e*), as has sometimes been assumed in the past.

KIMBALL, R. F., and NENITA GAITHER, Oak Ridge National Laboratory, Oak Ridge, Tenn.: *Effect of incomplete chromosome sets on behavior of nuclei at conjugation in Paramecium aurelia.*—Crosses were made between a diploid marker stock and clones having only haploid or both haploid and diploid micronuclei. Cytological examination shows that haploid micronuclei undergo meiosis at about the same rate as the diploid. Genetic evidence shows that segregation occurs in haploid meiosis so that the meiotic products have about half a chromosome set on the average. The aneuploids produced on crossing to diploids are inviable but frequently survive long enough to allow determination of the phenotype. When both haploid and diploid micronuclei are present in a common cytoplasm, the synkarya are derived almost entirely from products of the diploid; but this selective advantage is eliminated by a dose of about 5 kr of X-rays. The selection of complete meiotic products probably occurs during the entrance into the paroral cone of the one meiotic product which normally survives. The male gamete nucleus of the pure haploid functions successfully less often than the female. This difference probably results from a combination of two circumstances: (1) extra meiotic products sometimes survive outside the paroral cone, providing more than one source for the female but not the male pronucleus; and (2) the activities of the male gamete nucleus are more complex, allowing greater chance for loss. The nuclear behavior in these crosses is explained by autonomous effects of the deficient genome of individual meiotic products on the movement of these products in relation to the cytoplasmic regions of the conjugants.

KIRBY-SMITH, J. S., and DORIS L. CRAIG, Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee: *Some aspects of ultraviolet-induced chromosome aberrations in Tradescantia pollen.*—The induction of chromosome aberrations in Tradescantia dry pollen by ultraviolet radiation has been studied as a function of wave length, intensity, and dosage of applied radiation. Chromatid, isochromatid, and chromatid/chromatid interchanges have been observed with relative frequency of 1:0.5:0.05, respectively, for the three types. Chromatid and isochromatid aberrations have been produced in sufficient numbers to be studied quantitatively. An action spectrum for aberration production has been obtained which is in substantial agreement with the well-known curves for the bactericidal action and nucleic acid absorption of ultraviolet radiation. Aberrations are independent of intensity over a tenfold variation in dose rate. Exposures carried out in air and in nitrogen result in equal aberration frequencies for both chromatid and isochromatid types, although a significantly larger number of incompletely fused isochromatid aberrations are observed for pollen irradiated in nitrogen. This result may be explained in terms of an hypothesis that fewer primary breaks are produced in nitrogen, and that the breaks induced in material exposed in nitrogen are also less likely to rejoin than those produced in the presence of oxygen. Repeated experiments have failed to demonstrate any photorecovery of ultraviolet-induced breakage in dry pollen or in growing pollen tube cultures.

LAFONTAINE, JEAN, and HANS RIS, University of Wisconsin, Madison, Wisc.: *A study of lampbrush chromosomes with the electron microscope.*—Lampbrush chromosomes from oocytes of *Necturus*

maculatus, *Triturus viridescens*, *Pseudotriton montanus* and *Rana pipiens* were isolated and washed free of nuclear sap. They were fixed in 2% OsO_4, 1% neutral formalin or 70% ethanol, and dried with Anderson's critical point method. Stereoscopic electron micrographs were prepared which provide an excellent means for analyzing the 3-dimensional organization of these chromosomes. Ultra-thin sections of entire eggs, isolated nuclei and isolated chromosomes were used to study the details of "loop" and "chromomere" structure. The "loops" consist of a bundle of microfibrils each about 500Å thick. The microfibrils form helices of narrow pitch. The bundle as a whole is also coiled, forming a helix visible in the light microscope. Sections show that the 500Å microfibrils are split, each consisting of two thinner fibrils about 200Å thick. This double structure of the microfibrils is sometimes visible also in airdried shadowed preparations of "loops". The apparent central axis of the chromosome consists of dense bodies of various length and width, the "chromomeres". They appear structureless in whole mounts but in sections are seen to consist of microfibrils such as are present in the loops, but they are here more tightly packed. The loops issue from "chromomeres" and may return to the same one, though not to the point of origin, or they may return to the adjacent chromomere. No structure other than "loops" and "chromomeres", which both are made of microfibrils, were found. These observations are in agreement with the view that both "loops" and "chromomeres" are parts of the continuous chromonemata. Structurally they differ mainly in the arrangement (packing, coiling) of their component microfibrils.

LAUGHNAN, JOHN R., University of Illinois, Urbana, Ill.: *Intrachromosomal association between members of an adjacent serial duplication as a possible basis for the presumed gene mutations from A^b complexes.*—Noncrossover alpha derivatives from A^b ($\alpha\beta$) occur with a frequency of 10% that of the crossover alpha derivatives. The rate of occurrence of noncrossover alpha′ types from A^b:P ($\beta\alpha'$) is ten to twenty times greater. While their origin may be considered the result of gene mutation or spontaneous loss of the adjacent beta elements, the following considerations lessen the plausibility of such an explanation: (1) the alpha and alpha′ derivatives are phenotypically indistinguishable from the corresponding crossover isolates; (2) it is possible to consider spontaneous deficiency of the beta element as an explanation for these derivatives only if certain rigid assumptions regarding the localization, magnitude and time of occurrence of such deficiencies are invoked; (3) as in the cases of their crossover counterparts, the noncrossover derivatives occur individually, so far never in clusters, on the ear; this suggests that the crucial event in their origin occurs during or near meiosis and imposes limitations on the mutation and deficiency hypotheses; (4) preliminary evidence indicates that the frequency of occurrence of noncrossover alpha′ derivatives from A^b:P is influenced by the genetic constitution at the A locus in the homologue. An alternative explanation based on the evidence that the alpha- and beta-carrying segments correspond to a minute, tandem, serial duplication whose members have retained synaptic equivalence is proposed. It assumes that the adjacent members of the duplication may pair with each other in a double loop configuration at meiosis, an association which would be considered in competition with interhomologue pairing. Rare exchanges between the strands comprising the double loop would result in the loss and/or gain of one complete member of duplication, depending on the strands involved in the event. This mechanism would account for the rare individual occurrences of noncrossover alpha derivatives from the A^b complexes and appears to satisfy much of the evidence not easily accommodated by the hypothesis of gene mutation.

LEDERBERG, ESTHER M., University of Wisconsin, Madison, Wis.: *Pleiotropy for maltose fermentation and phage resistance in Escherichia coli K-12.*—Two loci, Mal_1 and Lp_2, have been independently described. Mal_1 controls maltose fermentation; Lp_2 determines resistance to virulent lambda mutants. Both were known to be linked to S (streptomycin) and usually hemizygous in otherwise heterozygous diploids. Recent evidence suggests that both effects are apparently pleiotropic manifestations of a single locus: 1) Existing stocks are either $Mal_1^-Lp_2^r$ or $Mal_1^+Lp_2^s$. 2) When these cultures were crossed, $Mal^+Lp_2^r$ and $Mal^-Lp_2^s$ crossover recombinants were absent despite extensive tests. 3) On EMB maltose agar $Mal^-Lp_2^r$ colonies gave Mal^+ reversions which proved to be Lp_2^s. In turn, $Mal^-Lp_2^r$ reappeared among the resistant survivors after exposure to the phage, lambda-2. This mutation cycle was repeated several times in a number of stocks. Occasionally mutants resistant

to lambda-2 were maltose-positive. These were tested by crosses with $Mal_1^-Lp_2^r$ and ascribed to mutation at any of several other loci. Genetically distinctive maltose-negative mutants have been found with unaltered phage susceptibility. The pleiotropy has also been noted in a second, probably nonallelic mutant phenotypically identical with $Mal_1^-Lp_2^r$ at 37° and $Mal_1^+Lp_2^s$ at 30°. The dual effects are therefore associated at two loci, and separated at others. All the *Mal*-mutants ferment glucose, and are presumably blocked in the single amylomaltase enzyme. Preliminary attempts to uncover the possible physiological basis of the correlation have been unsuccessful. The addition of either maltose or amylose to the medium had no influence on the phage reaction.

LEDERBERG, JOSHUA, and BRUCE STOCKER, University of Wisconsin, Madison, Wis., and Lister Institute, London, Eng.: *"Phenotypic" transductions of motility in Salmonella.*—The formation of genetically stable transformed clones by transduction of genetic fragments from one Salmonella strain to another has been well established for a wide range of markers, including motility. The possibility of scoring the motility of single cells has allowed the detection of a mode of transduction involving a determinant which is not regularly heritable. Motile cells were individually isolated within three hours of mixing a nonmotile strain with competent phage. Only five percent of the motile initials gave stably motile subclones, usually in mixture with co-segregant parental nonmotile cells. The remainder formed predominantly nonmotile clones containing no more than, at most, 100 motile individuals. The latter, when reisolated usually transmitted motility to only one daughter at each successive fission as if they carried a non-replicating determinant of motility. The exact nature of these determinants is debatable: they might be erstwhile genes that had been damaged or misplaced, or they might be persistent gene products or both. The nonrandom partition of motile progeny in subclones from early cell lineages suggests two levels of determination, either in organization or kind of motility-conferring "particles". The emergence of a motile phenotype and its transmission to a single chain of descent occurs spontaneously in a few cells of certain strains.

LEFEVRE, G., JR., M. M. GREEN, and P. C. FARNSWORTH, University of Utah, Salt Lake City, Utah; and University of California, Davis, California: *Reverse mutation at the forked locus in Drosophila melanogaster. X-ray induced mutation in the male germ line.*—The ability of a mutagen to produce reverse as well as direct mutations has long been recognized as evidence that the mutagen can induce "true" gene mutations rather than only destructive genetic changes. Since the original reports of the successful induction of reverse mutations by X-rays in Drosophila could not readily be confirmed by recent workers, the problem has been critically re-examined. The forked locus (*f*, 1–56.7) was chosen for study because it had been reported to revert more frequently than other loci tested. Several different suppressible and unsuppressible *f* alleles were investigated with respect to both spontaneous and X-ray induced reverse mutation. Two alleles, f^1 (suppressible) and f^{3N} (unsuppressible), were found to revert spontaneously: f^1 at a rate of about 1 per 50,000 gametes tested; f^{3N}, about 1 per 20,000. These spontaneous rates were observed in both the male and female germ lines, and in attached-X as well as in normal females. When f^1 and f^{3N} males were X-rayed with 5000r, reversions were found, but with a frequency equal to their respective spontaneous mutation rates. However, two other alleles, f^{36a} and f^{51a} (both unsuppressible), yielded neither spontaneous nor induced reversions. Apparently, X-rays induce no forked reversions in mature sperm; but the spontaneous reverse mutation rate of certain *f* alleles is so high that, in the absence of adequate control data, the results of X-ray experiments would suggest that reversions were being induced by the radiation. Other *f* alleles seem to be truly irreversible.

LESLEY, J. W., and MARGARET M. LESLEY, University of California, Citrus Experiment Station, Riverside, California: *The effect in R1, R2, and R3 of treating tomato seeds with X-ray and* P^{32}.—Seeds of inbred lines of tomatoes were exposed to 10,000r from X-rays or to beta radiation from radioactive isotope P^{32} in solution. Germination and growth was delayed and irregular. R1 plants especially from P^{32} often showed variegation and sometimes loss of pubescence. Similar changes occurred in R2. Some variegated R1 plants gave albino seedlings in R2. Soaking for 5 days in an initial dose of 10.8 microcuries P^{32} per seed had drastic effects. A few mature plants consisted of two or even more phenotypically and cytologically different parts. Much male sterility occurred in R1.

Male sterility associated with unfruitfulness was prevalent in R2 especially from unfruitful parents and fruit setting was delayed. Male sterility was frequently due to cytological causes. Both treatments caused in R1 translocations, interchanges, sometimes of unequal segments, inversions, weakened pairing, elongation and stickiness of chromosomes in meiosis. In R2 similar abnormalities occurred and, in addition, a few trisomics, homozygous deficients and duplications. Two variegated plants contained chromosome fragments. Mutants closely resembling known gene mutants occurred in R2, for instance after X-rays wiry (*w*) and after P^{32} yellow flesh of fruit (*r*) tangerine flesh (*t*) and anthocyanless (*a*), but the tangerine mutant unlike *t* was virescent and yellow-fleshed tended to be sterile. A new recessive mutant (*au*) occurred and apparent mutants for male sterility, time of fruit ripening, number of loculi in fruit and brachysm.

LEVINE, MYRON, Department of Bacteriology, University of Illinois, Urbana, Illinois: *The production of lysogeny for virulent bacteriophage by mixed infection.*—The wild type of phage PLT22 (ZINDER and LEDERBERG) of *Salmonella typhimurium* is a temperate, turbid-plaque phage, belonging to BOYD's group A. Infected bacteria are lysogenized in numbers that increase with the multiplicity of infection. Spontaneous clear-plaque mutants occur with a frequency of about 10^{-3}. These mutants can be classified into 3 groups, I, II, III. Mutants of group III are temperates showing low frequencies of lysogenization. Mutants of groups I and II, either individually or in mixed infection within the same group, produce only lytic responses with no lysogenization (= virulent mutants). Mixed infection with any mutant I and any mutant II results in the survival of many bacteria (up to 50%). These give rise to clones stably lysogenic for one of the infecting phage types. The phage liberated by these lysogenic bacteria is still virulent and almost exclusively group I. This novel situation of bacteria carrying the prophage of a virulent bacteriophage may account for natural reservoirs of virulent phages. Crosses between phages carrying characters I, II, or III (besides other markers) show that these characters segregate normally in the lytic cycle. Types I and III give equal frequencies of lysogenization in mixed infection, whereas in mixed infection of type II with either I or III lysogenization with type II occurs only exceptionally.

LEVINE, R. P., Harvard University, Cambridge, Mass.: *Cations in chromosome structure: Their relation to the mechanism of crossing over.*—Crossing over is significantly decreased in females of *Drosophila melanogaster* when as pupae they are subjected to extreme water loss at zero percent relative humidity. Cytological examination of the primary oöcytes reveals that the nuclei of desiccated animals have been reduced approximately eight times in volume in comparison with the nuclei of normal animals. In addition, alteration of the diet by the addition of excess calcium ions to the medium leads to decreased crossing over while the addition of a chelating agent results in increased crossing over. Cytological study has revealed that the nuclei of primary oöcytes of treated animals have been altered as to the intensity of their Feulgen staining compared with controls. The combination of these results is consistent with the idea that crossing over is sensitive to changes in the ionic environment of the chromosomes. This would be in accord with the supposition of MAZIA and the hypothesis of STEFFENSEN that a chromosome is composed of units linked in linear fashion through the chelation of calcium by terminal phosphate groups in DNA molecules. On the basis of such a model for chromosome structure and the results mentioned above, a mechanism for crossing over can be proposed in which it is assumed that genetic exchanges will occur at sites where calcium ions are presumed to be bound into the structure of a chromosome. (Work supported by an institutional grant of the American Cancer Society and by a grant (RG-4000) from the National Institutes of Health, Public Health).

LEWIS, HERMAN W., Massachusetts Institute of Technology, Cambridge, Massachusetts: *Genetic control of tyrosinase activity in Drosophila melanogaster.*—Comparisons have been made of the tyrosinase activity of extracts prepared from adult flies of the following genotypes: Canton-S (+); sable (*s*); suppressor-of-sable (*su*^{s}-*s*); and sable, suppressor-of-sable (*su*^{s}-*s s*). Sable is a sex-linked recessive gene associated with the production of a dark body color and is a temperature mutant. Homozygous sable females and hemizygous sable males raised at 19°C show the mutant effect, while mutant individuals raised at 25°C do not show the mutant phenotype. The *su*^{s}-*s* gene is a

sex-linked recessive and also suppresses the effect of the vermilion gene which controls eye color pigmentation. Although +, *s*, *su*^{s}-*s* and *su*^{s}-*s s* flies raised at 25°C are phenotypically indistinguishable, the enzyme activity of extracts from each of these genotypes is significantly different. The genotypes listed above are listed in the order of increasing enzymatic activity, the wild type extract being the least active and the suppressed sable extract having the highest tyrosinase activity. Using flies raised at 19°C the tyrosinase activity of the extracts of each of the genotypes is significantly increased although the enzyme activity of the extract of each genotype relative to the activity of the other genotypes remains the same. The evidence indicates that the activation of the enzymatic reaction for each of the genotypes is temperature sensitive and that the sable gene is associated with the production of a qualitatively different enzyme.

LIEB, M., Institut de Radium, Paris France: *Lysogenicity and immunity in Salmonella typhimurium.*—Lysogenic bacteria are immune to lysis by phage related to the carried phage. It has been proposed that prophage blocks the access of related phage to a nuclear site essential for initiating phage replication. *Salmonella typhimurium* lysogenic for phage A, STM (A), is immune to Ac, a non-lysogenizing mutant of A. After the exposure of sensitive STM to phage A at 37°, the number of bacteria lysogenized by A and the number immune to Ac were determined at intervals. The number of immunes and lysogenics is highest 2–6 minutes after infection and then decreases to a minimum at 20 minutes after infection. The percent of infected cells immunized and/or lysogenic is influenced by multiplicity of A, pH, and temperature before and after infection. At 37°, the number of lysogenics exceeds the number of immunes during the 25 minutes after infection with less than 5A per bacterium. Under certain conditions, the number of immune cells exceeds the number of lysogenics. This is attributed to a synergistic effect of Ac on lysogenization, and subsequent immunization, by phage A. At low multiplicities of A, bacteria about to divide are selectively immunized. A bacterium infected with a single A plus several Ac particles can divide to produce two immune bacteria in 10 minutes or less. However, loss of lysogenicity and immunity occurs in a small percentage of the progeny of subsequent divisions. Although the "site" notion is still partially tenable, immunity does not require the presence of a stable prophage.

LINDEGREN, C. C., E. SHULT and G. LINDEGREN, Southern Illinois University, Carbondale, Ill.: *Tetrad analysis in Saccharomyces.*—Tetrad analysis provides a level of information above single chromatid analysis for, in addition to the quantitative data revealed by single chromatid analysis, inspection of the three types of unordered tetrads yields certain specific qualitative information. When an excess of tetratype asci beyond random expectation is found it indicates that a high frequency of single exchange has occurred between the two markers involved. This occurs in the proximity of centromeres and is consistent with the cytological observation of localized chiasmata. Linkage of two genes to two different centromeres is qualitatively indicated when ascus types I and II are equal and the tetratype is less than two thirds of the total number of asci. Linkage to centromeres can be measured if four different loci are linked to four different centromeres; the distances from gene to centromere and gene to gene across the centromeres check additively. Other reported solutions of this problem involving three genes are illusory since the equations proposed yield solutions irrespective of the existence of centromeres. An interesting unusual situation first reported by VON WETTSTEIN is the occurrence of inverse linkage (which could naturally be detected as easily by single chromatid analysis). It is inferred that inverse linkage is the result of close linkage of two different loci to two different centromeres which undergo preferential segregation.

MARTIN, A., JR., VA Hospital, Leech Farm Rd., Pittsburgh 6, Pa.: *The Golgi bodies in tissue culture.*—Observations made on the Golgi substance indicate that it consists of a complex enzyme system containing a skeleton protein, and that this Golgi substance is active in the metabolism of fat. Tissues from representative vertebrates, prepared and examined according to conventional histological technique, indicate that lipids are directly absorbed through cell boundaries, and that the conversion of such lipids into living protoplasm within the cell takes place through the instrumentality of the Golgi substance. Observations on cells from oil-fed vertebrates grown and examined according to es-

tablished tissue culture technique indicate that the Golgi substance is active in the metabolism of fat, and that the Golgi substance varies in its activity from cell to cell in any given tissue and in relation to the physiological activity of a given cell. Tagged fat and phase optics have made it possible to follow the partition and distribution of the Golgi substance in mitosis, and to correlate data derived from tissue culture technique with those from histological technique. It has been possible to establish the Golgi substance as the seat of intracellular fat metabolism in representative vertebrates, and to follow the activity of this Golgi substance through several generations of cells. This would indicate a common gene structure for the Golgi substance morphology and physiology in the vertebrates, and further supports the hypothesis that many genes (some 70–80 percent of the genotype) are identical in structure and function in all living cells.

McCorquodale, D. James, and Robert E. Duncan, University of Wisconsin, Madison, Wisconsin: *The effects of imidazole containing compounds on the roots of Vicia faba and the reversal of these effects.*—Treatments of *Vicia faba* roots with 2.5×10^{-3} molar solutions of imidazole, benzimidazole or histamine in distilled water produce a complete inhibition of root growth, a sharp decrease in mitotic index, and a blackening of the roots except in meristematic regions. When, however, the roots are treated with 2.5×10^{-3} molar solutions of imidazole, benzimidazole, or histamine in complete mineral nutrient solution rather than in distilled water, root growth is not inhibited or only slightly so, the mitotic index is the same as in control roots, and there is no root blackening. It is therefore considered that imidazole, benzimidazole, and histamine produce their effects through interaction with metallic ions within the root cells. At present, it appears that the particular ion in the mineral nutrient solution responsible for the prevention of the effects of these compounds is either calcium or magnesium. All three compounds used contain the imidazole ring, a structural part of the purine components of nucleic acids. However, when roots of *Vicia faba* are treated with a 2.5×10^{-3} molar solution of the purine adenine in distilled water, no root blackening occurs, but root growth and mitotic index are inhibited strongly. This inhibition is not prevented by adding metallic ions. It appears, then, that the imidazole moiety of the purines does not act in the same manner as imidazole itself or the imidazole moiety of benzimidazole, or histamine. If, on the other hand, the purine ring is opened in such a manner as to leave the imidazole moiety intact, (as in 4-amino-5-imidazole carboxamide) then the imidazole containing compounds so formed act in a manner similar to imidazole, benzimidazole and histamine.

Mehlquist, Gustav A. L. and Yoneo Sagawa, University of Connecticut, Storrs, Conn: *The effect of gamma radiation on somatic mutation in the William Sim carnation.*

Melechen, Norman E., Dept. of Genetics, Carnegie Institution of Washington, Cold Spring Harbor, N. Y.: *The relationship of phage DNA synthesis to protein synthesis in replication of bacteriophage T2.*—Chloramphenicol is a specific inhibitor of protein synthesis in uninfected *Escherichia coli*. Its action on protein and DNA synthesis during phage reproduction was investigated. DNA and protein measurements were made by assaying P^{32} and S^{35} incorporation into the appropriate cellular fractions. In uninfected cells, addition of small amounts of inhibitor, up to 12–15 μg/ml, caused a decrease in the amount of protein synthesized to 3% of uninhibited controls. Higher concentrations up to 100 μg/ml, caused only a slight further decrease, to 0.5%. DNA synthesis continued at an uninhibited rate. In contrast to the behavior of uninfected cells, bacteria infected with T2 in the presence of 20 μg/ml of chloramphenicol formed neither protein nor DNA at appreciable rates. However, if an interval elapsed between the time of infection and the time of addition of chloramphenicol, DNA was formed subsequently at a rate determined by the length of the interval. After addition at 11 minutes, when DNA synthesis was just beginning, the rate in the presence of chloramphenicol was nearly equal to that of uninhibited cultures. DNA synthesis appears to be qualitatively independent of protein synthesis, but chloramphenicol-sensitive processes are required for the activation of DNA synthesizing mechanisms in infected bacteria. These conclusions are consistent with those reached by other workers using different systems. The specificity or role of this "activator" has not been determined. (This work was supported in part by a grant (C2158) from the National Cancer Institute of the Institutes of Health, Public Health Service).

MERICLE, L. W., V. R. PHELPS, and A. C. WHEELER, Michigan State University, East Lansing, Michigan: *Dose-effect relationships in X-irradiated barley embryos.*—Barley embryos *in situ* in varying stages of development (from time of fertilization through periods of differentiation until formation of the "mature" embryo as found in the seed) have been subjected to single doses of X-radiation varying from 50–800r. Samples for histological examination were taken at close intervals so that dose-effect relationships might be established. In general, the less mature embryos showed the greatest radiosensitivity (lowest threshold) with effects evident with as little as 200r. Nearly mature embryos may show little or no effect from as much as 500r. Threshold effects are conspicuous predominantly at a cellular level, consisting of a retardation of the normal rate of mitosis accompanied by a "compensatory" increase in cell size, increased cellular vacuolation, and, in general, a change from the more meristematic (embryonic) to a more mature (differentiated) condition. With higher dosages, X-radiation effects are evident at a tissue and organ level with developing root and shoot regions showing differential effects (root regions showing the most pronounced effects). With the higher ranges of radiation (500–800r) normal organogenesis may be so completely disrupted that the embryo is hardly recognizable as such, and, in fact, may abort, but if present is evident only as a mass of proliferating tissue. It is particularly significant that 200r of X-radiation if applied to very young embryos may produce more severe effects than 800r given to nearly mature ones.

MEYER, HELEN U., Indiana University, Bloomington, Ind: *Mutagenesis in the embryo of Drosophila melanogaster by antimetabolite fed to the parents and its apparent synergism with ultraviolet.*—Methionine sulfoxide ("M") and desoxypyridoxine hydrochloride ("D"),—two antimetabolites previously tested in combination with X-rays by OSTER—, were fed to adult Drosophila for one week (.2 and .1 g, respectively, per 50 cc of standard medium). Eggs were collected from these two treated lots, and from a control lot ("C"). In each experiment of this series, some eggs of all three lots were simultaneously irradiated with 200–300 ergs/mm² of ultraviolet (2537 Å), and others were not irradiated. Only the embryonic polar caps, containing the early germ cells, were exposed. Adults from exposed and unexposed embryos were bred and their offspring tested for chromosome-2 lethals by MULLER's "sifter" technique (D.I.S.25). The results, evaluated by special statistical methods (MULLER) gave the following harmonically weighted mean mutation rates: Non-irradiated groups: M, 0.58%; D, 1.6%; C, 0.33%. Irradiated groups: M, 4.9%; D, 8.8%; C, 4.3%. Thus, M-prefeeding proved ineffective, while the rates from irradiated lots D and C showed a significant difference ($P < .021$). The difference between unirradiated lots D and C was investigated further, with tests scoring sex-linked lethals, and a similar trend was observed: D, $.76 \pm .18\%$; C, $.36 \pm .13\%$. For the pooled data on non-irradiated lots D and C, a P-value of $<.016$ was found. Hence desoxypyridoxine hydrochloride if given in this manner is somewhat mutagenic, though found ineffective when injected into larvae (DI PAOLO). The results from the irradiated lots suggest that D and ultraviolet interact synergistically in mutagenesis. (This work has been supported by a grant received for work of H. J. MULLER and associates from the American Cancer Society, on recommendation of the Committee on Growth of the National Research Council.)

MICKEY, GEORGE H., Northwestern University, Evanston, Illinois: *Further analyses of position effects in Drosophila melanogaster.*—Among many chromosomal aberrations induced by radiations and being analyzed cytologically and genetically in our laboratory, stock No. 10c-2 is particularly interesting. This stock contains a reciprocal translocation between the Y chromosome and the right arm of the second chromosome, the break in the latter being very close to the brown locus. Males carrying both Y·2 and 2·Y chromosomes in the heterozygous condition show brown mottling or variegation of eye color. Males with Y·2 and two normal second chromosomes are phenotypically wild type but are sterile because they lack a part of the Y chromosome (they are hyperploid for the tip of 2R). Zygotes receiving the 2·Y chromosome in the homozygous condition do not survive either with a normal Y or with the Y·2. Occasional mottled females arise by non-disjunction of the X and Y·2 chromosomes in germ cells of 10c-2 males. Attached-X females also exhibit variegation when carrying both Y·2 and 2·Y chromosomes. Non-disjunction males can occur in crosses of 10c-2 males with variegated attached-X females. Such males carry both Y·2 and 2·Y chromosomes in the homozygous condition (also an X chromosome) and their eyes are almost solid brown with very slight mot-

tling. Interactions with eye color genes such as brown, brown dominant, and vermilion, as well as different environmental factors such as temperature and moisture conditions, and also varying amounts of heterochromatin in the genotype throw further light on the phenomenon of V-type position effect in Drosophila. (Work supported by U. S. Atomic Energy Commission grant—Contract No. AT(11-1)-89, Project No. 7.)

MILLER, W. J., and W. H. STONE, University of Wisconsin, Madison, Wis.: *Blood typing of the erythrocytes of bison with reagents for antigenic factors of cattle.*—Blood samples from 193 bison were recently obtained by courtesy of MR. E. J. GREENWALT from the Wichita National Wildlife Refuge, Cache, Oklahoma. These samples were tested with the same reagents (blood typing fluids) which are used to type the blood of cattle. All the bloods tested possessed antigenic factors either similar to or identical with V, L, X_1, X_2, X_3, and L′. There were 10 factors (A_1, H, J, S, Z, C_1, C_2, W, E'_2, and E'_3) which were present in some bison and lacking in others. These 10 factors represent specificities controlled by genes at 6 independent loci of cattle. Reagents for 24 other factors commonly found in cattle blood failed to react with the bison cells. The results of absorptions of the various reagents by reactive bison cells indicate that 3 factors (H, L′, and V) are similar but not identical in the two species. (This duplicates previous unpublished data for the factor V by C. STORMONT). Antisera for several antigenic factors of cattle were produced by immunization of rabbits with bison cells, and all of these anti-bison sera showed that bison cells contain one or more antigenic specificities not shared with the cells of cattle.

MOREE, RAY, State College of Washington, Pullman, Wash.: *A comparison of selection and inviability curves relating to experimental populations of Drosophila melanogaster.*—Four population cage experiments were started from the cross $+/e^{11} \times +/e^{11}$ and the decreasing frequencies of recessives from each cage were combined to give a single selection curve covering a period of 217 days. From supplemental experiments covering a range of population densities, coefficients of inviability were obtained and from these a theoretical selection curve was calculated for each density experiment, it being assumed as a working hypothesis that mating was random and that inviability coefficients were equal to selection coefficients. Since competition appears to approach a maximum in both population cages and supplemental experiments, it might be expected that all of the theoretical curves would have less slope than the experimental selection curve. Instead, the theoretical curves for the highest densities have greater slopes, and a theoretical curve can be constructed approximating to the experimental selection curve. Agreement between these curves cannot be taken as indicating, however, that selection is a consequence solely of inviability or of any of the other selection components. This would require, among other things, that competition in the population cage falls quite short of maximum, which seems unlikely. It is suggested that, in addition to inviability, these results depend in some way upon a balance between overlapping of generations and heterosis, which would decrease the effect of selection, and selective productivity of parents and selective mating on the other hand, which would increase it. (Work supported by grants from the National Institutes of Health and from the State of Washington Initiative Measure No. 171 for the support of biological and medical research).

MORSE, M. L., University of Wisconsin, Madison, Wis.: *Cis-trans position effect in transduction heterogenotes of Escherichia coli.*—The phage lambda can transduce a chromosome fragment which includes a cluster of genes for galactose fermentation. Most of the transformed clones are "diploid" or heterogenotic for the transduced genes. Many combinations of non-allelic *Gal*$^-$ mutants give galactose positive heterogenotes as readily as *Gal*$^+$/*Gal*$^-$. However, some combinations of *Gal*$^-$ gave smaller and delayed yields of positive clones. Further analysis disclosed a cis-trans position effect between certain loci. For example, while the cis $++/--$ heterogenotes formed by transduction from $Gal_1^+Gal_4^+$ to $Gal_1^-Gal_4^-$ are positive, the trans $+-/-+$ heterogenotes from the transduction from $Gal_1^+Gal_4^-$ to $Gal_1^-Gal_4^+$ are phenotypically galactose negative. In the negative clones, positive heterogenotes are later formed by crossing over in occasional cells. Further segregation results in all possible haploid combinations, $+-$, $-+$, $++$, and $--$. The delayed yields that were observed initially are based on these secondary events. Reciprocal transductions have given identical phenotypes, so that

in heterogenotes the genes in the fragment are functionally equivalent to the homologous genes in the chromosome. The galactose positive phenotype thus requires that + alleles be in adjacent positions either in the fragment or the chromosome.

MULLER, H. J., HELEN U. MEYER and E. A. CARLSON, Indiana University, Bloomington, Ind. *Further information concerning the multi-locus nature of the dumpy series in Drosophila.*—Different dumpy ("*dp*") "alleles" involve any combination of the following diverse effects: *o* ("oblique", shortened or "truncate" wings), *l* (lethality), *v* ("vortex", thoracic eruptions); compounds manifest effects common to both "alleles". On finding this "allelism", MULLER (1919–'23) based his inference that these diverse characteristics represent biochemically related effects of one complex gene, rather than a "gene-nest", on the following considerations. (1a) Some intensifiers of *o* intensified *v*. (1b) Mutation of a quite separate gene, *humpy*$^{+}$, gave a phenotype both oblique-like and vortex-like. (2) One of the three types of mutations combining two of the three effects must, on a three-locus interpretation, have involved two nonadjacent loci, skipping the middle. However, FOGEL (1949, these Records) proved the multiplicity of loci by obtaining crossover combinations. The following observations confirm and extend his discovery. First, *olv* has been obtained from *o/lv*, presumably by crossing over with *o* intensified by influence of *lv*. Second, this *olv*, by subsequent crossing over with $o^{+}l^{+}v^{+}$, reestablished *lv*. Distribution of markers *S* and *Sp* proved *o* leftmost here. Third, an ultraviolet-induced $ol^{(v)}$ (superscript indicating faint *v*) produced, from combination $ol^{(v)}/v$, clear cut $o^{+}l^{+}v^{+}$ crossovers. *S-Sp* distribution proved *v* rightmost here. A separate *l* locus remains unproved. Although phenotypic overlaps arose, the above cases proved genetic. Despite locus multiplicity (or doubleness), considerations 1a, 1b and reciprocal cross-influences (positional?) show that *at least* one pleiotropic gene, capable of mutating to change the characteristics differentially, does exist here. Several features of this case weaken consideration 2, however. They raise the possibility of some gene-mutations producing seeming rearrangements of the dumpy components. (Work supported by grant from American Cancer Society, on recommendation of Committee on Growth of National Research Council.)

NANNEY, D. L., University of Michigan, Ann Arbor, Mich.: *Mating type determination and inheritance in Tetrahymena pyriformis, variety 1.*—Seven mating types (I–VII) are known to occur in the strains designated as variety 1 of *T. pyriformis*, but inbred strains may differ in their abilities to produce some of the mating types. One series of strains (designated as Family A) produces in characteristic frequencies all types except IV and VII; another series of strains (Family B) produces all types except I. Breeding analyses indicate that the differences between the two series of strains are due to differences in a single chromosome. All seven types can be produced by the heterozygotes. Within an inbred strain, the differences between the mating types are due to persistent differences in the macronuclei. This conclusion is derived from the observation of caryonidal inheritance, very similar to that found in the Group A varieties of *Paramecium aurelia* but involving a larger number of mating types. Little or no correlation is observed between the mating type of a parental clone and that of its sexual progeny, nor among the various caryonides derived from a single conjugating pair. Macronuclear (mating type) differentiation is found to consist of a progressive elimination of potentialities, a process initiated at the time of origin of a new macronucleus and terminated at a later time with the establishment of a single pure mating type. The direction of the initial differentiation is influenced by the temperature at which the macronuclei develop. The final differentiation may occur within a few fissions if the cells are allowed to starve, or it may be postponed indefinitely if the cells are allowed to grow at maximal rate. (This work was supported by grants from the National Science Foundation.)

NEWCOMER, EARL H., The University of Connecticut, Storrs, Conn.: *Accessory chromosomes in the domestic fowl.*—Studies with a new technique on the sex and somatic cells of the fowl show six pairs of chromosomes which divide normally during mitosis and segregate in a regular manner in meiosis. In addition to these twelve well-defined chromosomes, there is a variable number of polymorphic, heterochromatic chromosome-like units which do not possess visible, localized kinetochores but which do divide, often allocyclicly, and perform the anaphase migration. In the maturation of sex cells, these

accessory units disappear by spermatid formation. They persist throughout the mitotic cycle but vary in number from cell to cell. They are stained with variable intensity by the Feulgen stain and vary in size from several micra to the limits of resolution of the microscope. Evidences of fusion and fragmentation are clear and this is supported by their variation in number and size.

NILAN, R. A., State College of Washington, Pullman, Wash.: *Post-radiation storage effect on chromosomes in barley seeds X-rayed at normally ineffective dosages.*—Dormant seeds of barley, variety Himalaya, which were X-rayed at 45r and 90r were stored in air and oxygen for 16 weeks. The frequencies of chromosomal bridges and acentric fragments were determined in shoot-tip cells of the seeds immediately after X-ray and at 6, 12, and 16 weeks after storage. Nonirradiated seeds were similarly stored and observed cytologically. Only three fragments and no bridges occurred in 500 cells of the control (non X-rayed and nonstored) seeds. This frequency was not altered by storage. The low dosages applied induced no aberrations as indicated by the fact that the aberration frequency in the irradiated seeds before storage was not significantly higher than in the nonirradiated seeds. However, there was an appreciable increase in aberrations in both 45r and 90r treated seeds after 6 weeks of storage and this frequency increased up to 16 weeks. In addition, the increase in aberrations was greater in the irradiated seeds stored in oxygen than in air. The most acceptable interpretation of the data is that potential or latent chromosome breaks are produced by the low dosages and that these breaks are transformed into actual breaks through the influence of oxygen during the storage period (rf. THODAY 1953, Heredity Suppl. 6: 299–310, and SWANSON 1955, Genetics 40: 193–203). Other possible interpretations will also be discussed. (This work has been supported by a grant-in-aid from the American Cancer Society on recommendation of the Committee on Growth of the National Research Council.)

OAKBERG, E. F., and R. L. DIMINNO, Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee.: *Gamma-ray sensitivity of different developmental stages of spermatogonia of the mouse.*—Hybrid 101 × C3H male mice were given γ-ray exposures of 5, 8, 12, 17, 23, 30, 38, 47, 57, 68, 80, and 100r when 12 weeks old. All animals were killed 72 hrs after irradiation. By this time, most degeneration of damaged spermatogonia has occurred, and comparison of number of normal cells in control and irradiated groups gives a reliable estimate of radiation damage. Classification of tubule stages on the basis of acrosome development, when combined with new refined estimates of the duration of spermatogenesis, allows determination of the stage at which spermatogonia were irradiated. Type B, intermediate, and type A spermatogonia in their third division are of comparable sensitivities as measured by primary spermatocyte production. Type A spermatogonia, however, are not of uniform sensitivity Irradiation just prior to their last division has less effect on cells destined to become dormant type A than on those which transform to the intermediate type. Also, heterogeneity of sensitivities is indicated for stages where all type A spermatogonia are in interphase. Thus markedly different responses of cytologically similar cells exist. The extreme sensitivity of spermatogonia to irradiation, with expression of the damage by cell death, demonstrates the severe selection which operates in experiments on genetic effects of irradiation of spermatogonia.

OBER, DOROTHY, University of Connecticut, Storrs, Conn.: *The genetics of variants of the William Sim carnation.*

OSTER, I. I., Indiana University, Bloomington, Ind.: *The induction of mutations in Drosophila melanogaster by orally administered ethyl carbamate (urethane).*—Previously RAPOPORT (1947) feeding Drosophila male larvae, VOGT (1948) injecting Drosophila adult males, and OELKHERS (1943) using Oenothera had found ethyl carbamate to induce mutations, including chromosomal aberrations. Some others have reported negative results. In studying the interaction between chemical and physical mutagens in *Drosophila melanogaster* (31st and 32nd Annual Report of the British Empire Cancer Campaign) it became evident that the offspring of urethane-fed males exhibited a high frequency of chromosomal alterations. Starved adult males were thereupon fed standard medium containing a 2.5M concentration of urethane. Offspring arising from sperm released 1–3 days after ter-

mination of a 10-day feeding-period were tested for chromosome breakage, lethal mutations, and translocations, using MULLER's (D.I.S.28) "multi-purpose" stocks. A corresponding group without urethane served as controls. In the treated series the frequency of X- or Y-chromosome loss or partial loss occasioned by breakage was 2.25% (34/1514) and the frequencies of lethals and translocations were 1.96% (9/459) and 2.73% (15/550) respectively, while the control group yielded 0.12% (2/1627) losses and 0.27% (2/738) lethals. Females free of preëxisting sex-linked recessive lethals were similarly treated and offspring arising from eggs laid 1–10 days after the cessation of treatment gave a frequency of 2.82% (10/355) lethals as compared with 0.37% (2/545) for the controls. These results confirm the report that urethane has a rather powerful mutagenic effect on male germ cells even when administered via the rather circuitous route of feeding, and show further its effectiveness in females, like what has been reported for mustards but unlike formaldehyde (AUERBACH, HERSKOWITZ). (Work supported by grant received for work of H. J. MULLER and associates from the American Cancer Society, on recommendation of the Committee on Growth of the National Research Council, and by a grant, from the British Empire Cancer Campaign, administered by C. AUERBACH.)

PARKER, D. R., University of Mississippi, University, Miss.: ***The origin of dominant lethals in irradiated oocytes of Drosophila.***—PATTERSON *et al.* (1932) showed aging of virgin females resulted in an increase in frequency of radiation-induced dominant lethals among the first eggs laid. A comparison has been made between radiation-induced dominant lethals and detachments of attached-X's in newly emerged adult females and those held virgin for five or seven days before irradiation and mating. The increase in frequency of dominant lethals involves a shift from a "two hit" to a "one hit" type of survival curve. Radiation induced detachment of attached-X's is markedly increased by aging, "two hit" curves being obtained with both aged and unaged cells. Furthermore, these detachments show the expected linkage with ends of other chromosomes. These findings invalidate the interpretation (LEA 1947; SONNENBLICK 1940) that dominant lethals induced in oocytes result from failure of breaks to rejoin until after fertilization. Since rejoining does occur prior to anaphase I, dominant lethals might result from the formation of anaphase II bridges (single anaphase I bridges being eliminated as in inversion heterozygotes). If the breakage in unaged oocytes is of single chromatids, two sisters must be broken independently to give rise to an anaphase II bridge, giving rise to a "two hit" curve. In aged oocytes perhaps these bridges result from sister union following isochromatid breakage. If the breakage in aged cells is primarily isochromatidal, this should yield a "one hit" curve. In either case, detachments of attached-X should remain "two hit".

PETERSON, PETER A., University of California, Riverside, California: ***Dosage effect on the mutability of a mutable pale green gene in maize.***—Mutable pale green (*pg*^{m}) mutates to the normal green allele (*Pg*) in the presence of an independently segregating dominant factor, Enhancer, (*En*). In the absence of *En*, *pg* remains stable, *pg*^{s}. The mutation, *pg*—*Pg*, is recognized by the appearance of green stripes of varying lengths (depending on the time of mutation) in pale green leaves. A comparison was made of the mutation rate (*pg*^{m}—*Pg*) in seedlings with 2 doses of the Enhancer gene (***En En***) versus seedlings with only 1 Enhancer gene present, (***En en***). The mutation rate can be obtained by counting the number of stripes in a specific area on the leaves of pale green seedlings. In families containing 2 doses of the Enhancer gene (*En En*), the mutation rate varied from 8.8 to 14.81 in the first seedling leaf and from 4.5 to 8.7 in the second seedling leaf. In families containing 1 dose of the Enhancer gene (***En en***), the mutation rate was approximately 31.1 in the first seedling leaf and over 27.5 in the second seedling leaf. In order to make a more direct comparison of the effect of 1 versus 2 doses of *En* within a similar background, the cross *En En* × *En en* was made. 2 different classes, *En En* and *En en* were obtained. Analysis of counts made on these leaves shows that the seedlings can be separated into two distinct groups, one high mutating and the other, low. Although the individual classes cannot be identified, the rates in the low mutating group correspond to the rates obtained in families where 2 doses of *En* were known to be present, while the rates in the high mutating group correspond to those of the *En en* families. In addition, measurements of the length of stripes, as indicative of time of mutation, show that the stripes on seedlings containing 2 doses (*En En*) are longer and subsequently occur earlier than the mutations in *En en* seedlings. Other studies in maize on the effect of varying the dosage of activators such as Dotted (*Dt*) and Activator (*Ac*), have shown

that the rate of mutation increases with an increase in dosage. In contrast to this, the mutation rate of pg^m as reported here decreases with an increase in the dosage of the controlling factor, Enhancer. This is similar to the results from recent studies with variegated pericarp where there is a very marked decline in mutation rate as the dosage of transposed Modulator (*Ir-Mp*) increases.

PITTENGER, THAD H., Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee: *Dominance relations in Neurospora heterokaryons.*—The dominance of the wild type allele of *pan* in Neurospora has been studied by using heterokaryons with controlled nuclear proportions. The growth rate at 30°C is normal when the proportion of pan^+ nuclei exceeds 3.5%. The growth rate decreases very rapidly with decreasing proportions of pan^+ nuclei in the range below 3.5%; e.g., at 2% pan^+ nuclei the growth rate is 20% of normal. The shape of the rate response curve is the same for added pan^+ nuclei as for pantothenate added to the medium; 2% of pan^+ nuclei is equal to about 0.2 μg of calcium pantothenate per ml. Suboptimal growth rates produced by disproportion of nuclear types are seldom constant over the length of a growth tube (300 to 400 mm), but are more uniform than the corresponding rates produced by limitation of pantothenate in the medium. The nuclear proportions change very little over 400 mm of growth, but there may be a very slow trend toward the establishment of nuclear ratios permitting more rapid growth. In a few cases the direction of the change was nonadaptive, however. It appears that the system controlled by the normal gene can function at a rate nearly 30 times that necessary for normal growth under the present conditions. It is not clear whether this extra capacity is a constant feature of the system or is evoked by the growth-limiting conditions, as, for instance, by an increase in the steady-state concentration of the precursors.

POULSON, D. F., Yale University, New Haven, Conn.: *Physiological genetic studies on copper metabolism in mutant strains of Drosophila melanogaster.*—Wild strains of *D. melanogaster* and its relatives (subgenus Sophophora) are able to live and reproduce on media containing as much as 200 μg Cu/g (POULSON 1950, Rec. Gen. Soc., 19, 118). Evidence has been presented that such strains are able to regulate their Cu uptake under certain conditions (POULSON, BOWEN, HILSE & RUBINSON 1952, Proc. N.A.S., 38, 912) while other species, e.g., *D. repleta* (subgenus Drosophila), cannot so regulate and accumulate Cu in proportion to the concentration in the medium over a wide range. These latter species cannot survive on high Cu concentrations, although through selection more resistant strains have been obtained. Studies in progress on mutant strains of *D. melanogaster* utilizing histochemical, radioisotope, and transplantation techniques indicate some differences from wild type strains with regard to uptake, localization, and excretion. Contrary to the report of KIKKAWA, OGITA, & FUJITA 1954 (DIS,28) strains carrying the body color mutant ebony, *e*, either alone, or in combination with other mutants such as *bw*, *cn*, *v*, *se*, and *dp* survive at the same high concentrations of Cu on which wild strains can be readily grown.

RABKIN, SAMUEL, Winter Park, Fla.: *Varied facio-jaw morphogenesis associated with normalcy or morbidity.*—In addition to prevailing physical dissimilarities, man is now experiencing an ever increasing trend towards facio-jaw morbidity and an expanding frequency in premature jaw bone age changes, viz. (a) structural malformations, mal-relations and deformities of the lower face and jaws; (b) morphologic inconstancy in pattern of the jaws and insufficiencies in bone formation that are invariably associated with premature pathologic jaw bone changes, presenescent degeneration.

Validly, the effects of modern civilization are primarily held responsible for the effective defects. Sequent factors are: nutrition, environment, childhood infectious diseases, endocrinopathies, and some less substantiated factors. Ostensibly, the above exert their influence during growth and development of the skeleton from birth to maturity.

Generally not included are: the early history of such known manifestations, the effects of random matings of contrasting physical types and defectives. Significantly, the element of constancy or morbidity as well as diverse skeletal morphology antedate modern man. Except for some incidental factors, physical and structural variations, normal or abnormal conditions, are predetermined with the first cell formation and are so exhibited in "cleared" embryo-fetus. X-rays of the more mature fetus corroborate these findings. (Some of the gathered evidence will be shown on slides.)

RAVIN, A. W., University of Rochester, Rochester, N. Y.: *Factors affecting the non-independent recovery of capsular and streptomycin-resistant transformants in Pneumococcus.*—Genetic transforming agents affecting synthesis of capsular polysaccharide and resistance to streptomycin can be found in DNA extracts of resistant, fully encapsulated pneumococci. The transforming activity of the two agents is dissociable. The incorporation of the capsular agent in a host cell (as evidenced by its phenotypic expression) requires that the dispersion of the cells in the medium be restricted. One means of restricting dispersion is through the use of agglutinating antisera. The incorporation of the agent for streptomycin-resistance does not exhibit the same dependence on agglutination. If the agents for streptomycin-resistance and capsular synthesis are obtained from different cells and combined before treatment of the host strain, the recovery of doubly transformed cells (i.e., both resistant and fully encapsulated) is not significantly greater than that expected were the two agents acting independently of each other. This is true in the presence or absence of agglutinins. A significant excess over the expected recovery of double transformants is readily observed whenever the cells are: (1) treated with the two agents present in the DNA extract derived from a single pneumococcal strain and (2) not subsequently agglutinated. The bearing of this finding on the question of *in vivo* organization of transforming agents will be discussed. (Research supported by National Microbiological Institute, U. S. Public Health Service.)

REBHUN, L. I., University of Chicago, Chicago, Ill.: *Nucleus-cytoplasm interaction in oocytes.*—Cytochemical studies of clam (Spisula) oocytes reveal dense, cytoplasmic bodies, usually juxtanuclear in position, containing, as indicated by Azure B-RNA-ase techniques, a several times higher concentration of RNA than the remaining cytoplasm. Electron micrographs of certain of these RNA containing bodies show layers of parallel double lamallae regularly arranged in three dimensions. In cross section and tangent view these lamellae have a morphology almost identical to that of the nuclear membrane. In tangent view the nuclear membrane shows annuli similar to those reported in other oocytes. In cross section the annuli appear as pores with amorphous material filling the pores. This similarity between nuclear membrane and cytoplasmic lamellae has also been found in the snail, Otala. Often lamellae are arranged many layers deep parallel to the nuclear membrane. Other cytoplasmic bodies can be related to these by a process of vacuolization. Often, this process seems to lead to hollow, basophilic bodies. Their interiors are occupied by granules similar in size and shape to the yolk platelets outside. These studies indicate the following sequence for the origin of the structures. RNA and other materials are organized in the nuclear-cytoplasmic interface into lamellae which then migrate into the cytoplasm. This occurs at localized areas of the interface in successive waves thus giving rise to stacks of lamellae. Once in the cytoplasm, vacuolization of the lamellae leads to other forms of basophilia apparently involved in yolk formation. If the RNA of these structures comes from the nucleus, this represents a major method of nucleus-cytoplasm interchange in oocytes.

REISNER, A., Indiana University, Bloomington, Ind.: *A method of obtaining specific serotype mutants in Paramecium aurelia stock 169, var. 4.*—Three autogamous (homozygous) animals, expressing ciliary antigen H, were isolated into three separate culture tubes. Enough culture fluid was added to allow each tube to reach a population of approximately 15,000 animals. The animals were then exposed to a 22 minute X-ray dose of 4,000r. Following irradiation, the population of each tube underwent three additional fissions before starvation to induce homozygosity through autogamy. The animals then underwent five fissions more in order to allow any block of antigen H to be expressed as a transformation of serotype away from H. At this point enough anti-169H serum was introduced into each of the three cultures in order to eliminate animals still of serotype H. The remaining 763 lines (approximately 255 per culture) were isolated and subjected to procedures known to induce serotype H in high frequency. Most lines eventually expressed H. Seven of the few remaining lines were crossed to a line isogenic with stock 169 but expressing serotype 29H (allelic to but distinguishable from 169H). The serotype H exconjugant hererozygotes expressed only 29H while the F_1 of a control cross of 169 normal $\times$ 29H isogenic expressed both 29H and 169H. Additional breeding analysis indicates that these lines differ from the normal at the same single locus, probably the locus controlling serotype specificity. Three of the H^- lines arose from different irradiated tubes and so have independent origin. The total number of haploid sets irradiated was about 180,000 and therefore the approximate mini-

mum mutation frequency at this locus equals 1.7×10^{-5}. (Work supported by a grant from U. S. Atomic Energy Commission (Contr. AT(11-1)-235).)

RIZKI, M. T. M., Yale University, New Haven, Conn.: *Spiracular glands in Drosophila.*—Three unicellular glands are associated with each posterior spiracle of *Drosophila melanogaster* larvae. These glands have branched cytoducts and each gland cell has a separate opening on the surface of the spiracle. The lipoidal nature of the secretion of these glands has been demonstrated with Sudan dyes and Oil Red on material embedded in Carbowax. Various acid and basic dyes, the Millon and periodic acid-Schiff reactions, amylase and pepsin have been utilized to study these glands.

RIZKI, M. T. M., Yale University, New Haven, Conn.: *Formation of the peritrophic membrane in Drosophila melanogaster.*—In *Drosophila melanogaster* the larval peritrophic membrane is secreted by a ring of cells located in the anterior region of the midgut "proventriculus" (cardia). This annulus, four cells in width, is differentiated from the remainder of the proventriculus by its heavier staining reaction with the periodic acid-Schiff reagent (PAS). The PAS-positive material of these cells as well as that of the peritrophic membrane is resistant to digestion by saliva, α-amylase, pepsin, trypsin, hyaluronidase, β-glucuronidase and RNAse. These cells cannot be distinguished from the adjacent cells with the use of Sudan B black, Millon, or acid and basic stains (fast green, naphthol yellow, toluidine blue, Azure B). Various pieces of larval midgut including or excluding this ring of cells have been implanted into adult males. These studies demonstrate that this ring of cells is necessary for the production of the material forming the peritrophic membrane (PAS-positive substance). Peritrophic membrane formation is abnormal in the embryonic lethal l(1)48J; PAS-positive material is accumulated in the lumen of the proventriculus at the level of this ring of cells. These cells provide favorable material for studying the intracellular synthesis of chitin. (Fellow of the American Cancer Society. Aided by a grant from the Sigma Xi-RESA Research Fund.)

ROMAN, H., University of Washington, Seattle, Washington: *Mutational studies in yeast.*—Ten independently-occurring recessive mutations of spontaneous origin have been obtained at the Ad_5 locus in Saccharomyces. The mutant cells require adenine for growth. At least four of the ten alleles differ from each other by the following test: Diploid cells containing two alleles of different mutational origin revert to adenine-independence with a frequency of about 1 in 10^7 cells; the reversion frequency is substantially lower if the two loci contain the same allele.

ROMAN, H., and H. C. DOUGLAS, University of Washington, Seattle, Wash.: *Selection for mutants affecting adenine synthesis in yeast.*—A haploid pink, adenine-requiring mutant strain of *Saccharomyces cerevisiae* provides the material for this study. Except for incidental markers, the mutant strain differs from the white, adenine-independent wild type at a single locus, the mutant carrying the recessive allele ad_2. When the pink strain is grown under aerobic conditions, new mutants arise and these accumulate at a rapid rate owing to their growth advantage over the ad_2 cells. The derived mutant cells are of two classes: they produce either white or pale-pink colonies. Of the derived whites, seven types have been identified. One of these is adenine-independent and is due to reverse mutation at the ad_2 locus. The other six are adenine-requiring and are the result of mutation to the recessive allele at five other loci. Their genotypes may be given as follows: $ad_2\ ad_3$, $ad_2\ ad_4$, $ad_2\ ad_5$, $ad_2\ ad_6$, $ad_2\ ad_7$, and $ad_2\ ad_5\ ad_7$. The latter is due to simultaneous mutation at the Ad_5 and Ad_7 loci and occurs more frequently than would be expected by chance. Ad_5 and Ad_7 are closely linked. The pale-pinks represent mutation to an intermediate allele at one or another of the five loci.

ROTHENBUHLER, WALTER C., and JOHN W. GOWEN, Department of Zoology and Entomology and Department of Genetics, respectively, Iowa State College, Ames, Iowa: *Chromosomal localization of the hereditary basis for gynandromorph production in honey bees (Apis mellifera L.).*—Sex-mosaic individuals were found in considerable numbers in a honey-bee colony in 1948. Three different, independent, genetic experiments have led to the conclusion that female parts of such gynandromorphs

develop by cleavage of the zygote, resulting from fertilization, whereas the male parts develop from one or more accessory sperms, which normally enter the bee's egg, but which normally degenerate. The tendency to produce gynandromorphs has been investigated in three ways with respect to its hereditary basis. (1) Mating females of selected gynandromorph-producing stock with males of selected gynandromorph-producing stock has increased gynandromorph production markedly. (2) Mating females of the gynandromorph-producing stock with males of non-gynandromorph-producing stock has resulted in gynandromorphs in the F_1 generation. Backcrossing F_1 females to males of the paternal stock through a series of generations has resulted in a complete loss of gynandromorph production in the third backcross. (3) Mating females of non-gynandromorph-producing stock to males of gynandromorph-producing stock has resulted in no gynandromorphs in the F_1 generation. Backcrossing F_1 females to the paternal stock through a series of generations has introduced gynandromorph production into the line by the fourth backcross. These results indicate that occurrence of gynandromorphic honey bees, in the stock under consideration, is due to inherited factors located, at least in part, in the chromosomes. Furthermore, it is the chromosomes of the mother, rather than those of the zygote, which regulate gynandromorph production.

RUDKIN, G. T., Institute for Cancer Research and Lankenau Hospital Research Institute, Philadelphia, Pennsylvania: *The ultraviolet absorption of puffed and unpuffed homologous regions in the salivary gland chromosomes of Drosophila melanogaster.*—Recent observations of morphological changes in puff-like regions of the salivary gland chromosomes at particular developmental stages (PAVAN and BREUER 1954; MECHELKE 1954) have stimulated interest in problems related to chromosome function. Cytochemical studies on the chromosomes themselves may be expected to elucidate chemical changes associated with this chromosomal activity. Variability in the expression of puffs in certain regions of the salivary gland chromosomes in late larvae and prepupae of *Drosophila melanogaster* makes it possible to study a single region in which a puff is present or absent. Ultraviolet absorption measurements were carried out on section 60 in chromosomes with and without puffs at subsections 60B to 60C. The measurements were carried out on photomicrographs taken at 257 mμ and at 275 mμ on calibrated photographic plates. The percentage of the measured area occupied by the puff region, used as an index of the degree of development of the puff, varied from 29 in unpuffed to 50 in puffed chromosomes. The percentage of the total extinction found in the same region varied from 31 to 33 at 257 mμ and from 29 to 34 at 275 mμ. The conclusion is reached that puff formation is not attended by a change in nucleic acid content, but that an increase in other substances having a low extinction in this spectral region, e.g. certain proteins, accompanies puff formation. (Aided by grant C1613 from the National Cancer Institute of the National Institutes of Health, U. S. Public Health Service, and an institutional grant from the American Cancer Society.)

SAEZ, F. A., and C. L. SOLARI, Departamento de Citogenética, Instituto de Investigación de Ciencias Biológicas, Montevideo, Uruguay: *Interspecific cytological variation in the genus Scotussa (Orthoptera: Cirlacanthacridinae).*—A cytogenetical study has been carried out of three species of this South-American genus of Orthoptera, whose area of dispersion is comprised between the 18° and 37° parallel in the southern hemisphere. *S. impudica* $2n♂ = 23$, has eleven pairs of acrocentric autosomes and an acrocentric X; *S. lemniscata* $2n♂ = 23$, eleven pairs of acrocentrics and an acrocentric X; *S. cliens* $2n♂ = 19$, one pair of metacentric autosomes, eight pairs of acrocentrics and an acrocentric X. Mean chiasma frequencies at metaphase in *S. impudica* is 14.4, in *S. lemniscata* 14.0 and in *S. cliens* 11.9. Chromosome polymorphism has been found in *S. lemniscata*, of the seven males studied three possessed a supernumerary chromosome, having 24 elements instead of 23. The reduction in chromosome number from 23 to 19 in *S. cliens*, should have arisen in two different ways: (a) formation of a metacentric chromosome (constantly present in the homozygous state) by centric fusion of two rod-shaped autosomes, (b) loss of one pair of autosomes of the median length type. One individual of *S. cliens* was apparently heterozygous for a paracentric inversion judging by the presence of bridges and small fragments in some cells. If we consider that the ancestral caryotype of the genus *Scotussa* is $2n♂ = 23$, we have to assume that the evolutionary change in *S. cliens* took place secondarily.

SAGER, RUTH, The Rockefeller Institute for Medical Research, New York, N. Y.: *Non-Mendelian inheritance in Chlamydomonas.*—A non-Mendelian pattern of inheritance of streptomycin resistance in a strain of *Chlamydomonas reinhardi* was previously described. It was shown that resistance is transmitted either to all offspring of particular crosses, or to none; and that the ability to transmit resistance exhibits 2:2 segregation. (Proc. Natl. Acad. Sc. 1954). The results of a further analysis will be reported: (1) The same pattern of inheritance has been found in 8 independently isolated streptomycin resistant strains of both mating types. (2) The ability to transmit resistance has been demonstrated to depend upon mating type, as shown directly by reversal of transmission following mutation of mating type and indirectly, by various crosses. (3) Exceptional sets of progeny segregating for resistance and sensitivity have been recovered from individual zygotes, providing evidence for the particulate nature of the non-Mendelian factor.

SCHALET, A., Indiana University, Bloomington, Ind.: *The relationship between the frequency of nitrogen mustard induced translocations in mature sperm of Drosophila and utilization of sperm by females.*—Other workers have found after chemical treatment fewer interchromosomal rearrangements in Drosophila sperm than after X-ray treatment, when doses yielding equal frequencies of recessive lethals were compared. Furthermore, storage of chemically treated sperm in males or untreated females did not significantly influence induced lethal rates. Present experiments show that after treatment of sperm in females an increase in translocation frequency is observed in sperm released during successive periods. Following postcopulatory vaginal douches of females the frequency of sex chromosome loss (partial or complete), X-chromosome recessive lethals, and translocations was determined for 0.25% nitrogen mustard by MULLER's "multi" technique. Mutations were scored separately in offspring derived from successive periods of egg laying (1st–6th day, 7th–9th day, 10th day on). Losses involving either paternal sex chromosome were: 0.8% (9/1092); 0.4% (2/530); 1.0% (5/498). Lethal rates were 5.9% (56/947); 5.2% (24/458); 6.3% (27/432). Translocation frequencies were: 0.8% (7/860); 2.4% (11/454); 8.3% (32/384). Evidence was obtained that the increase in translocation rate was not attributable to translocations in maternal chromosomes. All sperm chromosomes tested participated. The distribution of translocations obtained from these and similar tests utilizing nitrogen mustard (II-III 31, Y-II 6, Y-III 25, and Y-II-III 8), differed, with respect to the ratio of II-III as compared with Y-II and Y-III translocations combined, from the ratio obtained after triazine treatment (HERSKOWITZ 1955, these Records), and X-ray treatment (MULLER, HERSKOWITZ and OSTER unpublished) of sperm in females, but resembled the ratio obtained by neutron treatment of sperm in males (MULLER 1954). (This work has been supported by a grant received for work of H. J. MULLER and associates from the American Cancer Society, on recommendation of the Committee on Growth of the National Research Council.)

SCHEINBERG, S. L., University of Wisconsin, Madison, Wis.: *Linkage groups of cellular antigens in the chicken.*—Data from testcrosses indicate the existence of two independent linkage groups composed of genes for cellular antigens in the fowl. A recombination frequency of 2.6% among 111 progeny was obtained between the genes in one linkage group. In another independent linkage group, a recombination frequency of 5.4% for 228 progeny was obtained between the two linked genes. The antisera which detected the antigenic products of the linked genes were produced by immunization of chickens and rabbits with chicken erythrocytes. These antisera were then differentially absorbed to obtain specific reagents. Serological crossreactivity was not observed between the antigenic products of the genes in either linkage group.

SCHWARTZ, D., Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee: *Mutations at the yg_2 locus in maize.*—The mutagenic effects of ionizing radiations are being investigated by use of the pseudoallelic series of chlorophyll markers on the short arm of chromosome 9. MCCLINTOCK has shown that the yg_2 locus is located on the first chromomere just proximal to the knob. Closely linked on either side are the two more extreme chlorophyll markers, *Wd* and *Py*. Plants of the constitution *Wd* Yg_2 *Py/wd df*, heterozygous for an irradiated (1000r) normal chromosome 9 and one deficient for the terminal 6 chromomeres, were scored for mutations at the Yg_2 locus. Plants having

such mutations would show a yellow-green phenotype. Minute deficiencies which deleted only the Yg_2 locus are indistinguishable from true gene mutations of Yg_2. Deficiencies which include the *Wd* or *Py* loci in addition are excluded by virtue of their giving rise to white or pale yellow seedlings. The results do not show any significant increase in yellow-green mutants from the irradiated material over the unirradiated control. These studies do not provide support for the contention that transformation of genes to allelic forms is accelerated by ionizing radiations.

SCOTT, J. P., Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine: *The genetic analysis of thresholds affected by both hereditary and environmental factors, with particular reference to behavioral characters.*—Early geneticists tended to neglect the analysis of characters showing a large variability due to environment, but this cannot be avoided in behavioral characters. A method of analysis is described based on the assumption that a threshold exists, dividing the subjects into two classes. It is assumed that genetic and environmental factors summate at the point of the threshold. The difference between the proportions of one class in each pure breeding parent stock may be considered as due only to heredity, and the relative position of segregating stocks is given according to the number of genes involved. The relation $Bx_1\text{-}Bx_2/P_1\text{-}P_2$ is particularly useful, since it is not affected by possible heterozygosis in a one factor cross. The SE of the expected fraction may be calculated as the SE of a difference. The method is applied to several behavioral characters, and its use compared with WRIGHT's method of analysis of variance for analyzing quantitative characters.

SCOTT, D. MCN., E. D. DELAMATER and E. J. MINSAVAGE, University of Pennsylvania, Philadelphia, Pa.: *Synchronization of metabolic events during synchronous division in Escherichia coli and Bacillus megaterium.*—The increases in DNA and RNA content of the bacteria, and the utilization of glucose have been determined at frequent intervals during division.

SEARS, E. R., United States Department of Agriculture and University of Missouri, Columbia, Missouri: *An induced gene transfer from Aegilops to Triticum.*—The chromosomes of *Triticum aestivum* (n = 21; genome formula ABD) were combined with those of *Aegilops umbellulata* (n = 7; C^u) by hybridizing *T. aestivum* with the amphiploid *T. dicoccoides* (n = 14; AB) × *Ae. umbellulata*. The resistance of *Ae. umbellulata* to leaf rust, *Puccinia triticina*, proved to be epistatic to the susceptibility of the wheat material. After two backcrosses to *T. aestivum*, a resistant plant was obtained with the full 21 pairs of wheat chromosomes and a single Aegilops chromosome. This chromosome had a deleterious effect on the plant and on pollen performance as well. Plants with an added derived isochromosome involving the resistance-carrying arm were X-rayed previous to meiosis, and later were used to pollinate normal, untreated plants. Of the 6091 offspring, only 132 were resistant, and 50 of these had a translocation involving the Aegilops chromosome. At least 16 different translocations were represented, most being reciprocal translocations. One translocated chromosome, which evidently had an Aegilops centromere and two doses of resistance, showed by its pairing behavior that the resistance gene is located near the centromere. Each reciprocal translocation therefore involved almost the entire arm of the Aegilops chromosome. Male transmission of resistance from heterozygotes was only about 5%. Four translocations had higher male transmission rates, ranging from 27% to 43% and suggesting that these four were intercalary rather than reciprocal translocations. The one with 43% transmission has no detectable effect on the plant except to confer resistance and to delay maturity slightly under some conditions.

SINGLETON, W. R., and A. L. CASPAR, Brookhaven National Laboratory, Upton, New York: *Effect of chronic gamma-radiation on somatic mutations in maize.*—Corn plants of the genetic constitution P^{RR}/p were grown at different distances from a Co^{60} source. The radiation received by the plants varied from .8r/day to 185r/d. However, at the highest radiation level plants made a poor growth and produced no seed, with few seed produced at 103–160r/d. Dose response curves were calculated for radiation from 1.0r/d to 46r/d for orange, variegated, and trace mutants (those showing only a fine streak of mutant character); also for total mutations greater than trace for doses from 0.8r/d to 94 r/d. There was an inverse linear relationship between seed set and radiation received, varying from approximately 550 seeds per ear at 1.0r/d to 130 at 94r/d. Further reduction resulted from higher

doses but populations were too small to make satisfactory estimate of seed set. Mutations greater than trace showed a linear relationship for the higher doses of radiation but no increase over control in % of mutations below 5r/d. More than a million seeds were examined. Mutations involving a kernel or more were rare and showed little increase with increasing dosage. All of these were variegated. The orange mutants showed an apparent linear relationship at doses in excess of 8r/d with a different slope to the curve below 8r/d. Trace mutants showed similar relationship. These data indicate a difference response for the low and high level radiation. They also indicate most mutations occurred late in the ear development and very few of the mutants were large enough to subject to a genetic test. (Work carried out at Brookhaven National Laboratory under the auspices of the U. S. Atomic Energy Commission.)

SKAAR, P.D., and ALAN GAREN, Biological Laboratory and Carnegie Institution, Cold Spring Harbor, N. Y.: *Transfer of DNA accompanying genetic recombination in E. coli K-12.*—The cell to cell passage of DNA was assayed by mixing bacteriophage resistant cells labelled with P^{32} with unlabelled phage sensitive cells, lysing the sensitive cells, and measuring the radioactivity in the DNA of liberated phage. DNA transfer was found to be predominantly unilateral, from Hfr to F−, and directly correlated with genetic transfer as inferred from recombination analyses.

SMYTH, J. ROBERT JR., University of Massachusetts, Amherst, Massachusetts: *Selection for differing levels of sexual receptivity in the female turkey.*—In the domestic turkey the initiation of a mating is primarily determined by the female. When approached by a male, a receptive female assumes a characteristic crouch, thereby inviting a mating attempt. It has been previously shown that females differ markedly in the frequency with which they accept a male and that the level of sexual receptivity in the female is an important component of fertility in the turkey (SMYTH and LEIGHTON Poul. Sci. 32: 1004–1013, 1953). In order to determine to what extent, if any, sexual receptivity in the female turkey is determined by genetic factors, selection for high and low mating frequency lines was initiated in 1951. An evaluation of each individual female's mating behavior was made by limiting the male's presence in the breeding pen to a daily one half hour observation period. Six observation periods each week were made for a seven week period starting just prior to the onset of the mating period. In the fourth selection generation the high mating frequency females averaged 10.7 matings per hen for the seven week period, as compared with a mean of 4.8 matings for the low line females. The present mean difference between the lines is due largely to an increase in the sexual receptivity of the high line. These data indicate that this important component of fertility has a heritable basis and will respond to selection.

SONNEBORN, T. M., and MYRTLE V. SCHNELLER, Indiana University, Bloomington, Ind.: *Genetic consequences of aging in Variety 4 of Paramecium aurelia.*—The older the parent clone (in fissions since the last fertilization), the higher is the frequency of origin of non-viable and weak clones at autogamy, until all are non-viable from parents 250 fissions old or older. On the contrary, mortality at autogamy remained uniformly low (about 0.7%) in 50 successive autogamies at about 25 fission intervals in an unselected line of descent. The high mortality at autogamy in old parents is thus not due to a steady rate of accumulation of genetic damage, but is a specific effect of old age itself. That this effect is genetic and localized in the germinal nuclei (micronuclei) was demonstrated by (1) obtaining "merogones" with young nuclei in old cytoplasm and (2) obtaining true hybrids between young and old. In both cases, normal vigorous offspring were obtained, although the old parents were unable to produce viable progeny by autogamy. The F_2 from the old × young vigorous hybrids gave high percentages (up to 100%) of non-viable progeny and some visible "mutants". Breeding behavior and cytologic observations indicate that these results are due to the production of frequent chromosomal aberrations in the germinal nuclei of old clones. In this respect, old age has effects similar to irradiations. (Work supported by grants from U. S. Atomic Energy Commission (Contr. AT(11-1)-235) and the American Cancer Society, on recommendation of Committee on Growth of National Research Council.)

SONNENBLICK, B. P., Newark College of Arts and Sciences, Rutgers University, Newark, N. J.: *Considerations concerning the X-ray dose received by the human ovary during certain diagnostic procedures.*—There has been much discussion recently concerning the possible genetic influence of radioactive fallout. Added to the natural radiation background, fallout can at present average levels increase an individual's exposure by approximately 0.1 to 0.2r over a reproductive cycle of three decades. We shall present intravaginal measurements made with thirty women undergoing diagnostic procedures used in obstetrics and gynecology; these are X-ray pelvimetry (films taken prior to labor and involving fetal exposure) and hysterosalpingography (films for fallopian tube studies). Furthermore, the ovarian dose received during abdominal fluoroscopy can be estimated from our measurements of roentgen output of a large number of fluoroscopes. These are common procedures, differing from fallout in that only a portion of the population is implicated. From 2–4r can be delivered to the ovaries within minutes, much more to the skin where readings were also made. This constitutes an appreciable addition to the total natural radiation background level received by an individual woman during an entire reproductive cycle. The only other comparable estimate for hysterosalpingography (in England) is strikingly higher than our measurements. These low doses bring to mind the probable although not directly proven principle of a non-threshold dose effect. They pointedly suggest a difficult but necessary systematic study of genetic effects on a multicellular organism of low doses (10–12r or less), given acutely and chronically, including the subsequent influence of these exposures on populations of the test organism. Despite the required extrapolation of experimental data to man, the recommended investigations could provide information significant for the evaluation of radiation effects.

SPIESS, ELIOT B., and BROTHER ROBERT J. SCHUELLEIN, University of Pittsburgh, Pittsburgh 13, Pennsylvania: *Chromosomal adaptive polymorphism in Drosophila persimilis from high elevation in the Yosemite region.*—The population cage technique was used to calculate net adaptive values of the Whitney and Klamath arrangements of chromosome III of *D. persimilis* taken from a locality at 8000 feet elevation in Yosemite Nat'l. Park. The results indicate that the WT/KL heterokaryotype is superior to WT/WT homokaryotype which in turn is superior to KL/KL. Egg laying capacity, viability, and rate of development were studied in detail employing several replications of each karyotype under optimal and crowded conditions. Fecundity of WT/WT females is not significantly different from KL/KL in the period tested; WT/KL derived from WT mothers is uniformly higher by about 3 eggs per day while WT/KL derived from KL mothers is much lower in certain combinations and much higher in others. Viability (percent hatchability of eggs plus percent survival of larvae and pupae) is much greater for WT/KL than for the homokaryotypes which are equal. Rate of development is much faster for WT/WT than for the other karyotypes by about 4 days at 15°C under optimal conditions. Under crowded conditions (higher egg and larval densities) viability of heterokaryotypes is still superior to homokaryotypes. These facts indicate that heterosis for loci on the third chromosome is expressed in terms of survival ability in the stages from egg to adult but not for fecundity or for rate of development. The superiority of WT/WT over KL/KL in population cages may be accounted for in part by its faster rate of development. WT is the most common arrangement of chromosome III in the locality sampled, and these results show that the factors tested can account in part for the observed frequencies of these inversions in nature. (Work supported in part by a grant from the U. S. Atomic Energy Commission (Contract AT (30-1)-1775).)

STADLER, JANICE, and JOHN W. GOWEN, Iowa State College, Ames, Iowa: *Localization of factors responsible for genetic resistance to Salmonella typhimurium in mice through X-ray analysis.*—Inbred lines of mice displaying characteristic degrees of resistance to typhoid infection, *S. typhimurium*, have been differentiated in the course of our genetic studies. These strains when exposed to 200,000 organisms line 11C, intraperitoneally inoculated have the following percentages of survival in recent tests S 99.7, RI 93.1, K 74.4, Z 73.7, E 24.0, L 23.2 and Ba 0.2. Ability to resist weight changes following irradiation and during the course of the disease; heart, kidney, and liver size; blood volume and hematocrit percentages; particular serum albumins and globulins; lymphocyte numbers; ability of liver cells to isolate disease tissue and allow uninjured cells to function in glycogen and fat storage;

ability of phagocytic cells to ingest and digest bacteria were all factors in this complex of interactions among the host constitutions affecting disease progenies under irradiation. On the other side, genetic weight prior to irradiation or infection seemed unimportant. Spleen sizes, despite their pronounced reactions to the disease and irradiation, were not indicative of strain resistances. Exposure of the mouse to radiant energy reduces its resistance to mouse typhoid. Study of mice exposed to X-rays, where the irradiations were limited to particular regions, has confirmed the importance of the above organs in the genetically controlled resistance of the different strains. (This work has received assistance from contract No. AT(11-1)107 from the Atomic Energy Commission.)

STEBBINS, G. L., and G. D. MCCOLLUM, University of California, Davis, California: *The induction of genic and chromosomal sterility in a subspecies hybrid of Dactylis by irradiation of pollen.*—A plant of the diploid form *Dactylis glomerata* subsp. *lusitanica*, was pollinated with pollen from another diploid, subsp. *judaica*, the pollen having been irradiated with 1250r. Out of 126 F_1 plants which were raised, 51 had less than 20 percent of normal pollen. Seed fertility of these plants ranged from 0 to 60 percent. Among 43 individuals of an F_1 progeny of subsp. *lusitanica* × *judaica* made with non-irradiated pollen, the range was from 38 to 88 percent of normal pollen. Among 120 plants of a progeny obtained by pollinating the same plant of subsp. *lusitanica* with pollen of another plant of subsp. *lusitanica* which had been irradiated with 1250r, only 5 plants were found with less than 20 percent of normal pollen. At meiosis the majority of the *lusitanica* × *judaica* plants with low pollen fertility form seven bivalents in at least some sporocytes, but some were found which form one ring or chain of four chromosomes, and a few had two rings or chains of four. Four of the sterile plants were treated with colchicine to double their chromosome number. Two of the induced tetraploids were fertile. One of these was derived from a diploid with slightly irregular meiosis but little obvious structural hybridity, while the other was derived from a diploid heterozygous for two translocations. The other two induced tetraploids remained as sterile as the diploid sectors of the same plant. In one of these sterility was clearly due to marked disturbances of meiosis initiated at I anaphase.

STEFFENSEN, DALE, Brookhaven National Laboratory, Upton, N. Y.: *Interaction effects of metal ions in the production of spontaneous chromosomal aberrations in calcium-deficient Tradescantia.*—*Tradescantia paludosa* (clone 5 of SAX) grown in water culture with a Ca deficiency showed at least 17 times the frequency of chromosomal fragmentation found in plants grown on optimal Ca when observed in microspores or pollen. Numerous collections and large samples have demonstrated the non-random nature of the production of chromosomal aberrations with low levels of Ca. In single cultures buds of different inflorescences fixed at the same time have mean frequencies of micronuclei which differ by 10–14 fold. At the low Ca concentrations employed high Mg or Mn appeared neither to replace Ca nor have an antagonistic effect, and low Mg exhibits little if any enhancement effect. With optimal available Ca, however, significant increases in the number of micronuclei occur in Mg-deficient pollen. In an Mg-deficient culture with twice optimal Ca the number of breaks tended to be lower. The Ca-deficiency effect appeared to be reduced when K was suboptimal. This reduction could be explained by a $K \rightleftarrows Ca$ antagonism, where in presence of low K, Ca is more easily transported from roots to inflorescences. A hypothetical model for the chromosome is postulated, which corroborates and extends a recent suggestion of MAZIA and other biological and physical-chemical data, that Ca forms chelate bonds with the terminal phosphate groups of different DNA "species". The chromosome is considered, at least in part, to be a giant chelate-polymer. At one "locus" DNA molecules of the same "species", which have exactly the same length, would be coordinated in precise juxtaposition by cations. Cross-linking by cations would occur between two phosphate groups of different molecules at each revolution of the double helix. (Research carried out at Brookhaven National Laboratory under the auspices of the U. S. Atomic Energy Commission.)

STIMPFLING, J. H., and M. R. IRWIN, University of Wisconsin, Madison, Wisc.: *Some evidence for chromosome homologies in five species of doves, genus Streptopelia.*—Backcross hybrids have been obtained by mating to *Streptopelia risoria* (Ring dove) the species hybrids and selected backcross hybrids from matings of *St. chinensis*, *St. humilis*, *St. orientalis* and *St. senegalensis* to *risoria*. Anti-

genic substances of the erythrocytes which distinguish each of these species from *risoria* are determined either by single genes or by two or more genes which to date have not separated by crossing over. The following antigenic characters are considered here: d-1 of *chinensis*, hu-1 of *humilis*, or-1 of *orientalis* and s-1 of *senegalensis*. The specific reagent for each antigenic character is cross-reactive with the other three characters. By appropriate procedures it has been demonstrated that each substance has an antigenic property or properties which distinguishes it from the remaining three substances. Hence, these four unit characters from the four parental species are serologically similar, but no two are identical. A cellular antigen of *risoria*, designated r-1, has been demonstrated to be antithetical to d-1, or-1, s-1 and is presumably antithetical to hu-1. Therefore, d-1, hu-1, or-1 and s-1 should also be contrasting substances with varying degrees of relationship. From these interrelationships among the four antigenic characters there may be inferred varying degrees of relationship among the chromosomes which bear the causative genes.

ST. LAWRENCE, PATRICIA, Yale University, New Haven, Conn.: *Investigations of the Q locus in Neurospora.*—An attempt has been made to analyze the genetic relationships between four mutants of separate origin which differ from wild type in loss of a known biochemical reaction. These mutants require niacin, accumulate quinolinic acid, and do not form heterocaryons; they are considered to represent blocks of the same synthetic step in niacin formation. The mutants *q-1*, *q-2*, and *q-3* give rare spontaneous reversions and are located in linkage group I, approximately one unit distal to lysine (4545). Reversions in *q-4* have not been obtained, and it has not been possible to procure evidence for recombination of this mutant and lysine. Mutant *q-3* shows persistent reduced crossing over in a region proximal to lysine. Stocks of the mutants carrying various arrangements of markers were crossed and the ascospores plated in appropriate media but without niacin. Colonies which appeared were isolated and tested by crossing to wild type. 90% or more of the colonies tested contained a niacin-independent nucleus. Such colonies were obtained only from crosses between mutants; each pair of mutants gave characteristic frequencies of niacin-independent colonies. Additional tests revealed that neither the presence of an extra chromosome nor the occurrence of suppressors could account for these niacin-independent colonies. The distribution of linked markers among these isolates indicated that they do not arise by crossing over. It is suggested that the origin of niacin-independence may be related to a mutational process involving a restricted chromosomal region associated with the *q* locus. (Project supported in part by the American Cancer Society on recommendation of the Committee on Growth.)

STONE, W. H., and W. J. MILLER, University of Wisconsin, Madison, Wis.: *Alterations in the reactivity of cattle erythrocytes following treatment with enzymes.*—The major alterations in reactivity following treatment of cattle erythrocytes with solutions of trypsin or papain appear to be restricted to antigenic factors produced by genes at only 2 or 3 of the 11 independent loci effecting cellular antigens. The most noticeable effects following trypsin-treatment are losses in reactivity for several antigenic factors (Q, Y_2, E'_1, E'_2, and T_2) of the B-system irrespective of the complex of antigenic factors. On the other hand, some factors (Y_1, E'_3, I', T_1 and O_3) of the B-system may or may not be affected, apparently depending upon the complex in which the factor occurs. In the C-system the factor L' is always inactivated by either trypsin or papain. In contrast, the reactions of cells containing the factor B are sometimes enhanced following trypsin-treatment. Also, some but not all cells, ordinarily non-reactive with the H-reagent became definitely H-positive after treatment with trypsin. These results show that enzymes may effect hemolytic reactions as well as agglutination reactions.

STRAUSS, BERNARD S., Department of Zoology, Syracuse University, Syracuse 10, New York: *The nature of the lesion in the succinate-requiring mutants of Neurospora crassa: Interaction between carbohydrate and nitrogen metabolism.*—Mutants of *Neurospora crassa* requiring dicarboxylic acids for an immediate growth response (*suc* and *at suc*) oxidize acetate and are inhibited by fluoroacetate with consequent citric acid accumulation to approximately the same extent as the wild type. This indicates use of the tricarboxylic acid cycle for acetate oxidation in organisms unable to grow with

acetate as a growth supplement. The concentration of nitrogen (as ammonium and nitrate salts) present in the conventional growth medium is inhibitory to the growth of these mutants and leads to an accumulation of acetylmethylcarbinol and keto acids. This inhibition is reduced and growth is stimulated by the addition of dicarboxylic acids or by diminution of the nitrogen present in "minimal" medium. The amount of carbon dioxide fixed by wild type conidia is increased by over 450% in the presence of nitrogen; *suc* conidia show an increase of less than 50%. It is likely that the dicarboxylic acid deficiency in the *suc* mutants is the result of a lesion in the mechanisms of carbon dioxide fixation. The addition of nitrogen salts to *suc* mutants probably diverts dicarboxylic acids (already in limited supply) from the catalysis of the oxidation of C_2 fragments via the tricarboxylic acid cycle to other reactions. This effect of nitrogen salts upsets the already precarious dicarboxylic acid balance of the *suc* mutants leading to a growth requirement and to the accumulation of intermediates. (Supported under contract AT(30-1)1138 between Syracuse University and the Atomic Energy Commission.)

STURTEVANT, F. M., Div. of Biol. Res., G. D. Searle & Co., Chicago, Ill.: *Stathmokinetic effect of a kojic acid derivative in the Allium test.*—A series of kojic acid derivatives was tested for stathmokinetic activity on the dividing cells of root tips of *Allium cepa* var. Yellow Multipliers (Vaughan). Onion sets were rooted in tap water and transferred to 5×10^{-6} M aqueous solutions of the compounds for treatment periods of 0, 3, 6, and 24 hours. Root tips were removed at these times, fixed in HCl, stained with acetocarmine, smeared, and examined microscopically. The most active compound in the series was SC-5148, 2-(5′-hydroxy-4′-oxo-2′-γ-pyranylmethyl)-2-thio-3-methlypseudourea HCl. This compound produced numerous c-pair mitoses, many metaphases, but few or no anaphases. These disturbances were present as soon as 3 hours after the start of treatment. By 24 hours, a minority of anaphases appeared, many of them abnormal. In this test, SC-5148 was more active than podophyllotoxin. (SC-5148 was synthesized by DR. PETER KRIMMEL of these laboratories.)

SUSKIND, S. R., C. YANOFSKY, and D. M. BONNER, Yale University, New Haven, Conn.: *An immunochemical study of allelic strains of Neurospora lacking tryptophan synthetase.*—An immunochemical study of mutant strains of *Neurospora crassa* unable to form the enzyme tryptophan synthetase has been undertaken. Immunization via a combination of serial and adjuvant injections with partially purified enzyme preparations can effectively elicit the formation of antibodies against *N. crassa* tryptophan synthetase. Preliminary data have been obtained on the immunochemical characterization of the tryptophan synthetase-anti-tryptophan synthetase reaction. Antibody against partially purified tryptophan synthetase preparations was found to neutralize enzyme activity. Extracts of several allelic tryptophan-requiring mutants, which lack the enzyme tryptophan synthetase, were examined for the presence of proteins which cross-react with tryptophan synthetase antibodies. All of the mutants studied were found to possess such material except one. The cross-reacting material in the mutants followed the same course of purification as tryptophan synthetase. Mutants which carry specific suppressor genes, with the attendant formation of low levels of tryptophan synthetase activity, were found to form both the active enzyme and cross-reacting material. The relationship of these cross-reacting antigen or antigens to tryptophan synthetase is not yet known. However, the presence and absence of proteins antigenically related to tryptophan synthetase in different members of a group of very similar mutants may well suggest that those differences reflect defects in specific and separate phases of the synthesis of tryptophan synthetase.

TAYLOR, J. H., and R. D. MCMASTER, Columbia University, New York, N. Y.: *Studies on nucleic acid metabolism in larval salivary glands of Drosophila.*—By means of autoradiographs, the incorporation of P^{32}-inorganic phosphate and C^{14}-glycine into nucleic acids and proteins of cytoplasm, chromosomes and nucleoli has been studied. P^{32} is most rapidly incorporated into the RNA of certain sectors of the chromosomes and into the nucleolus, and more slowly into the cytoplasmic RNA of salivary gland cells of early third instar larvae. Glands fixed by the freeze-substitution method show intense basophilia of nucleoli and cytoplasm after staining with Azure B at pH4. Microphotometric determinations of Azure concentrations have indicated equal concentrations of RNA in the nucleoli and

cytoplasms of the cells which were used for incorporation studies. Since the RNA concentration is no higher in the nucleolus than in the cytoplasm, the higher radioactivity of the nucleolus indicates a higher rate of incorporation per unit of RNA. Glycine is incorporated into both RNA and proteins during the same period of development, but the difference between nucleoli and cytoplasm is much less striking. Since the ratio of C^{14} in protein to that in RNA is high, the more uniform pattern of incorporation found with this isotope may be due to protein rather than to RNA. Significant variations in RNA-C^{14} could be obscured by the high concentration of protein-C^{14}. Autoradiographs and stained slides showing localization of radioactivity and basophilia will be demonstrated. Techniques of preparing autoradiographs for the collection of quantitative data, and graphs of the course of incorporation will be shown.

TSAI, L. S., Mount Holyoke College, So. Hadley, Mass.: *Embryological abnormalities in Drosophila melanogaster caused by two deficiencies for the brown locus.*—The study of two deficiencies for the brown locus, symbol *bw*, on the second chromosome of *Drosophila melanogaster* show that both the homozygous *Df(2)bw^{vDe2}* and *Df(2)bw^{5}* affect the embryonic development producing an incomplete extension of the head fold. The effects of the former deficiency seem to occur earlier in development, as the dorsal closure of the midgut is never completed. The absence of the mid-dorsal hypoderm makes the formation of the normal head fold impossible. As a result no definite mouth armature is produced. In the case of *Df(2)bw^{5}*, the head fold extension stops at a later stage of development. Only the anterior end of the cephalopharyngeal region (about ¼ of the whole) is uncovered. A definite mouth armature is produced but it is abnormal in form. Neither of these types of abnormal embryos can hatch. The lethal effects of the homozygous *Df(2)bw^{vDe2}* and *Df(2)bw^{5}* embryos show certain similarities to other embryonic lethals in Drosophila, *Df(2)vg^{C}*, *Df(2)vg^{D}*, fused, Krüppel and Minute IV, but each of these appears to show a specific pattern of damage.

WEIR, J. A., University of Kansas, Lawrence, Kansas: *Use of artificial insemination to evaluate the influence of the sire on sex ratio in mice.*—Differential loss of embryos between implantation and sex classification, in polytocous species, provides the usual explanation of anomalous sex ratios. Discovery, from reciprocal crosses, that the characteristic sex ratio of two lines of mice (pHH—55%♂♂, pHL—45%♂♂) is completely determined by the male parent, points to pre-fertilization influences on X and Y sperm. If, for example, a ♀ of low sex ratio line (pHL) is mated to a vasectomized ♂ of like strain, and then artificially inseminated, by laparotomy, with sperm from the ductus deferens of a freshly killed ♂ of the high sex ratio line (pHH), the sex ratio of the litter may be expected to follow one or the other ♂ line. If the sex ratio is influenced by the "sterile semen" donor it would seem that differential survival of sperm normally occurs after insemination. If the sex ratio is determined by the sperm donor, it would seem that the selective effect is exerted during or soon after maturation. Cumulative results from artificial matings of the various possible combinations, along with results of natural matings, will be presented. (Supported by grant [G-3141] from the National Institute of Health, U. S. Public Health Service.)

WELSHONS, W. J., Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee: *A comparative study of crossing over.*—Data have been collected which yield information on the process of crossing over as it occurs in attached-X females of *Drosophila melanogaster*. In one case parents were heterozygous for a third chromosome inversion, and in the other case the inversion was absent. The attached-X chromosomes were heterozygous for six closely linked recessive markers. Crossover products emerged in females with a wild phenotype and were recognized by progeny testing. There are two types of single crossovers, reciprocal and nonreciprocal exchanges. If single exchanges occur at random these two types should be found in equal numbers. In the absence of the autosomal inversion, there was no departure from the expected 1:1 ratio. The total exchange frequency was 298 reciprocal: 306 nonreciprocal. When the autosomal inversion was present, the two types of exchanges still occurred at random. The frequency was 181 reciprocal: 179 nonreciprocal. Two types of double exchanges may be recognized. Type-1 doubles result from a 2-strand double and a 3-strand double exchange. Type-2 doubles result from a 3-strand double and a 4-strand double exchange. In the absence of chromatid interference, type-1 and type-2 doubles should be found in

a ratio of 1:1. When the inversion is not present, the ratio is 63 type-1:59 type-2. In the presence of the inversion, the ratio is 30 type-1:25 type-2. These data indicate that the reduction in X-chromosome interference under the influence of an autosomal inversion cannot be a reflection of an altered chromatid interference.

WHITING, ANNA R., and SARAH CASPARI, University of Pennsylvania, Philadelphia, Pa., and Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tenn.: *Time of death of embryos with dominant or recessive lethals induced by X-rays in Habrobracon eggs.*—Unmated females were X-rayed with 1100r. Some were bred unmated, others were mated to untreated males. Eggs which had been irradiated in first meiotic metaphase were used. Those which failed to hatch were fixed and whole mount Feulgen preparations made. Surviving daughters of the mated females were bred as virgin and hatchability of their eggs recorded. Where it was 50% or lower, inviable embryos were handled as described above. Dominant or recessive lethals kill embryos (haploid) from unfertilized X-rayed eggs; dominant lethals only, those (diploid) from eggs X-rayed and then fertilized; recessive lethals only, those embryos (haploid) in eggs from unmated F_1 females. Dominant lethal changes kill the majority of embryos during the first three hours of development. Recessive lethal changes kill almost all the embryos twelve or more hours after development begins. The few early deaths are comparable in frequency with those in controls. Dominant lethals tend to kill embryos at later stages in fertilized than in unfertilized eggs.

WHITING, P. W., University of Pennsylvania, Philadelphia, Pennsylvania: *Linkage relations of purple, glass and the eye-color locus R in Mormoniella.*—The allelic series *R* is linked with purple, *pu*, body color, giving 10–15% crossovers. The eye shape glass, *gl*, gave no recombinations with *pu* among 2750 F_2 males (*R* not involved). Tested with *R* in various combinations (*pu* not involved) *gl* gave parentals,—2180 *gl*, 2383 gl^+ and crossovers,—41 *gl*, 28 gl^+. With three points involved there were 13801 *pu* or *gl* with parental eye color, 322 (158 *pu*, 164 *gl*) with reversed eye color and 10 (6 gl^+ pu^+, 4 *gl pu*) with parental eye color following *pu*, reversed for *gl*. There were no recombinations between *gl* and *pu* in which parental eye color followed *gl*. These data show total recombinations between *gl* and *pu* (10/16883) 0.06%, between *gl* and *R* (401/18765) 2.14%, between *pu* and *R* (322/14133) 2.20%. Heterozygosis for *gl* shortens map distance between *R* and *pu*. It is suggested that the order of the genes is *R* − *pu* − *gl* and that *gl* is within an inversion suppressing crossing over between *R* and *pu*, that this inversion does not include *R* but may or may not include *pu*. (Work supported by U. S. Atomic Energy Commission, Contract AT (30-1)-1471).

WOLFF, G. L., National Cancer Institute, Bethesda, Maryland: *The Harding-Passey melanoma as a bio-indicator of some metabolic effects of the ob gene.*—A technique developed by WOODS, WRIGHT, HUNTER and BURK (Biochim. et Biophys. Acta 12: 329–346) to determine the effects of a stress-accentuated insulin-anti-insulin hormonal system on the anaerobic glycolysis of transplantable mouse melanomas has been adapted to detect some of the metabolic effects of the obese (*ob*) gene in the mouse. Previous work by MAYER and others has indicated that the *ob* gene exerts at least a pleiotropic effect on the hormonal balance of the mouse. By implanting the Harding-Passey melanoma in normal and gonadectomized non-obese (*Ob-*) and obese (*obob*) ♂♂ and ♀♀ and determining the rates of anaerobic glycolysis of the tumor *in vitro* in the presence of exogenous insulin, testosterone, and insulin + testosterone, it has been possible to gather evidence confirming that a hormone imbalance induced by the *ob* gene does indeed exist. The extent and rather complex relationships of this evidence will be illustrated with charts. (U. S. Public Health Service Post-doctoral Fellow of the National Cancer Institute.)

YANOFSKY, C., and D. M. BONNER, Western Reserve University School of Medicine, Cleveland, Ohio and Yale University, New Haven, Conn.: *Non-allelic suppressor genes affecting a single td allele.*—Previous studies of the 25 *td* mutants of *Neurospora crassa* have shown that they are all blocked in the conversion of indole to tryptophan, they are all defective in tryptophan synthetase formation, they all appear to be allelic, and that certain members of this allelic series respond to the presence

of allele-specific suppressor genes by forming tryptophan synthetase and growing in the absence of a tryptophan supplement. The present study was undertaken to determine whether or not all suppressor mutations affecting a specific *td* allele would be at a single locus. Strain td_2 was irradiated with ultraviolet light and 10 slow growing tryptophan independent colonies (presumably carrying suppressors) were selected for further study. Suppressor genes affecting strain td_2 were detected in cultures derived from 4 of these colonies. The suppressor genes were designated su_{2a}, su_{2b}, su_{2c} and su_{2d}. The results of appropriate crosses indicated that none of the 4 suppressor genes was allelic with su_2, a previously studied suppressor of td_2. In addition, the new suppressor genes were shown to be at separate loci with the possible exception of su_{2b} and su_{2c}. Whether or not these 2 suppressor genes are allelic has not as yet been established. It appears from these findings that a specific defect in tryptophan synthetase formation can be overcome by mutation at any one of a number of different suppressor loci, despite the extreme allele specificity of these suppressor genes.

YERGANIAN, G., and C. LIVINGSTON, Children's Cancer Research Foundation, Inc., Boston, Massachusetts: *Sensitivity of tumor chromosomes to colchicine.*—Arrest of metaphase chromosomes in Sarcoma CH-38MC, induced in the Chinese hamster, *Cricetulus griseus*, by methylcholanthrene, has provided further clarification of the random distribution experienced by each of the 11 morphologically distinguishable chromosome types. Pretreatment of tumor tissue for 30 minutes (5 μg/15 grams tissue) resulted in properly spaced and moderately contracted bipartite chromosomes with exceptionally clear primary constrictions: an extremely valuable criterion in any analysis of mammalian karyograms. Previously reported phenomena displayed among untreated cells of the 20th passage (Proc. Am. Assoc. Cancer Res. 2: 56, 1955) were found to persist in the present study of the 24th transplant generation: (a) absence of 1, 2, or 3 specific chromosome types from apparently viable tumor cells, (b) accumulation of as many as 6 representatives of a given chromosome type in "diploid" and "aneuploid" cells, and (c) approximately equal division rates for cells having 23 to 27 chromosomes, regardless of the presence, absence, or duplication of any one of the 11 types of chromosomes. Failure to reveal a stable and replicable stem-line genotype, as described for mouse and rat tumors by other workers, serves to illustrate the broad heterogeneity that can result in rapidly proliferating malignant tissues. The tumor stem-line concept remains inapplicable to this particular tumor. Repeated non-disjunction during bipolar divisions of "near-diploid" and multipolar divisions of "hypertetraploid" cells is regarded as the major factor leading to chromosome variegation in tumor cells. (Work supported by a grant from the Damon Runyon Memorial Fund for Cancer Research, Inc.)

EFFECTS OF PERICENTRIC REARRANGEMENTS ON RECOMBINATION IN GRASSHOPPER CHROMOSOMES

M. J. D. WHITE AND F. H. W. MORLEY

Division of Plant Industry, C.S.I.R.O., Canberra, Australia

Received January 8, 1955

THE significance of chromosomal rearrangements of various kinds in the adaptive polymorphism of many animal and plant species depends to a large extent on their effects on crossing over. In Drosophila species, crossing over does not normally occur in the male, so that we are only concerned with the effects of rearrangements on crossing over in oogenesis. The evidence (MORGAN, BRIDGES and SCHULTZ 1930; STURTEVANT and BEADLE 1936; STEINBERG 1936, 1937; KOMAI and TAKAKU 1940, 1942; STEINBERG and FRASER 1944; DOBZHANSKY and EPLING 1948; SCHULTZ and REDFIELD 1951; CARSON 1953) has been almost entirely genetic, cytological studies being technically too difficult in this material. Among the effects of heterozygosity for paracentric inversions in Drosophila the following are well-established; (1) almost complete suppression of effective crossing over within the limits of the inversion, due in part to elimination of crossover strands in the polar body nuclei, (2) suppression or drastic reduction of crossing over outside the inverted region, both proximally and distally, (3) an increase in the amount of crossing over in the other members of the chromosome complement (the "Schultz-Redfield effect"). Most of those who have studied these phenomena seem to consider that the second type of effect extends as far as the ends of the chromosome limbs, but KOMAI and TAKAKU (1940, 1942) have reported an actual increase of crossing over near the distal end of the *Drosophila virilis* X chromosome when two heterozygous inversions were present in the proximal region.

Highly complex systems of chromosomal polymorphism are now known in many species of grasshoppers, and more particularly in those belonging to the North American group Trimerotropi (see WHITE 1954a, pp. 148–155 for a general review). The most characteristic type of rearrangement in this group has the effect of converting an acrocentric chromosome into a metacentric element. Such rearrangements are accordingly pericentric; whether they are actually inversions is still uncertain, but appears probable (a detailed study of the pachytene bivalents is needed to settle this point).

Rearrangements of this type are not confined to the group Trimerotropi. They occur, for example, in certain races or populations of the Australian grasshoppers *Cryptobothrus chrysophorus* and *Austroicetes pusilla* and in at least four species of the genus Moraba, which belongs to a different family of grasshopper-like insects (the Eumastacidae). As previously pointed out (WHITE 1951) such rearrangements, when heterozygous, almost completely suppress chiasma formation in the segment between the two centromeres of the bivalent, so that hardly any chromatids with duplications and deficiencies are produced at meiosis, at any rate in the male. Thus the regions which are structurally heterozygous are genetically isolated and no gene-transfer can occur between them.

The present study represents an attempt to determine more precisely the general effects of these pericentric rearrangements on chiasma formation, and hence on genetic recombination, in the chromosome segments *outside* the limits of the rearrangement. Since in grasshoppers chiasmata are formed in both sexes it would be necessary to have data from both oogenesis and spermatogenesis in order to present a complete picture; but for technical reasons it is hardly practicable to carry out a cytological study on the oocytes and the investigation was accordingly limited to spermatogenesis.

MATERIALS AND METHODS

It was important for the present purpose to study chromosomes that could be recognized from cell to cell and from one individual to another. Out of several dozen cases of 'heteromorphic' chromosomes available for study, four were selected for detailed analysis, one in *Trimerotropis sparsa* (material from various localities in Utah and Nevada), two in *T. gracilis* (material from the Humboldt Range, Nevada) and one in *Moraba scurra* (material from Blundells, Australian Capital Territory). In addition, some studies on the effect of different numbers of heterozygous rearrangements on the amount of recombination in the entire chromosome complement were carried out on Californian material of *Trimerotropis suffusa*. All the material of *Trimerotropis* spp. was sectioned, but in the case of *Moraba scurra* both sections and squash preparations were employed.

The Sevier rearrangement in Trimerotropis sparsa

On a number of alkali flats in western Utah and eastern Nevada there live populations of *Trimerotropis sparsa* in which almost every individual has one or more chromosome pairs structurally heterozygous (WHITE 1951). One rearrangement, in the largest chromosome, which is particularly recognizable, has been called Sevier. The Standard first chromosome is acrocentric, while the Sevier one is a metacentric with two rather unequal arms. The appearance of *St/St*, *St/Sev* and *Sev/Sev* bivalents with various numbers of chiasmata is shown in figure 1. It will be noted that the *St* chromosome usually shows a conspicuous constriction very close to its proximal end, which separates an ovoid satellite from the main body of the chromosome. It is uncertain whether this is actually the centromeric constriction, i.e. whether the satellite represents the second arm of the chromosome. Occasionally, the constriction and satellite are not visible, and whenever this is so the condition is constant throughout the individual (a bivalent in the middle of the top row in figure 1 is heterozygous for the absence of the satellite). Probably a minute rearrangement affecting the centromere region is present in some individuals in heterozygous or homozygous form. This rearrangement is, however, fairly uncommon in the populations studied and there is no reason to believe that its presence or absence affects the chiasma frequency of the bivalent. No constriction can be seen in the centromere region of the *Sev* chromosome at first metaphase.

The number of chiasmata in the first bivalent was counted in 14 *St/St*, 23 *St/Sev* and 9 *Sev/Sev* individuals (table 1), the total number of bivalents examined being 4168. Most of the individuals were collected at a locality approximately 5 miles SE of Cherry Creek, White Pine Co., Nevada, but a few of them came from Milford,

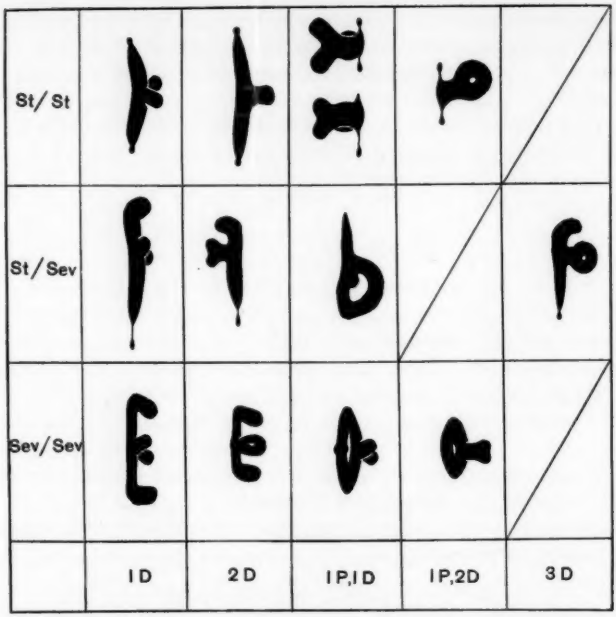


FIGURE 1.—The various appearances assumed by the largest bivalent of *T. sparsa*, according to whether it is heterozygous or homozygous for the Standard and Sevier sequences and according to the number of chiasmata in the distal and proximal regions.

TABLE 1

Chiasma frequency data on the Sevier rearrangement in T. sparsa†

Type of bivalent	Individuals number and location*	0 prox. 1 dist.	0 prox. 2 dist	0 prox. 3 dist.	1 prox. 0 dist.	1 prox. 1 dist.	1 prox. 2 dist.	2 prox. 0 dist.	2 prox. 1 dist.	Mean Prox.	Mean dist.	Total bivalents
St/St	14 CC	74	96	—	24	860	60	1	3	0.875	1.105	1118
St/Sev	16 CC + 4 M	1441	784	6	—	3	—	—	—	0.001	1.374	2234
	3 B	309	9	—	—	25	—	—	—	0.069	1.025	343
Sev/Sev	8 CC + 1 M	54	35	—	3	326	55	—	—	0.798	1.108	473

* CC indicates a locality 5 miles S.E. of Cherry Creek, White Pine Co., Nevada; M indicates Milford, Utah; B indicates a locality near Beaver, Utah.

† Complete data on file at the GENETICS Editorial Office.

Utah and three individuals, which will be discussed separately, were collected at Beaver, Utah.

In table 1 the terms 'proximal' and 'distal' are used to designate the regions of the Standard chromosomes corresponding to the short and long limbs of the one carrying the Sevier rearrangement. There is, of course, no difficulty in deciding whether a

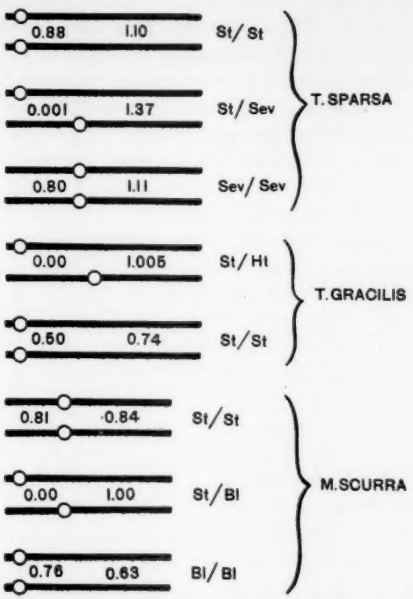


FIGURE 2.—Diagram showing the chiasma frequencies of the proximal and distal regions of the various types of bivalents studied in *T. sparsa*, *T. gracilis* and *M. scurra*.

chiasma is in the 'proximal' or 'distal' segment of the *St/Sev* or *Sev/Sev* bivalent. In the case of an *St/St* bivalent, however, there is nothing to mark the boundary between the two regions and the scoring of chiasmata as proximal or distal is less objective. Since, however, the great majority of the chiasmata lie clearly in one region or the other, only a small number lie so close to the boundary that any real doubt exists. A few errors of assignment have probably been included in the data, but these must have been far too few to affect the validity of the conclusions reached. A study of diplotene bivalents showed that there is no appreciable terminalization of chiasmata except in the case of ones very close to the chromosome ends.

Considerable heterogeneity between individuals was evident in the data. Thus in the case of *St/St* individuals the chiasma frequency of the proximal region ranged from 0.640 to 0.970 and that of the distal region from 0.850 to 1.393. In the case of the *Sev/Sev* individuals the corresponding ranges were from 0.489 to 1.000 and from 1.000 to 1.257. Such variation from one individual to another is characteristic of chiasma frequency data. It is shown, for example, in MONTALENTI's (1947) Simulium data, in CALLAN and MONTALENTI's (1947) data on various species of mosquitoes, and even in the grasshopper *Bryodema tuberculatum* with extreme chiasma localization (WHITE 1954b). It is not known to what extent this variation results from genetic causes or is due to environmental influences (age may be an important factor).

In figure 2 the average chiasma frequencies of the proximal and distal regions of

St/St, *St/Sev* and *Sev/Sev* bivalents are shown (these frequencies are the means of the separate means of individuals). In calculating the means for the *St/Sev* bivalents the data for the three individuals from Beaver have been omitted, for reasons explained later. The analysis of variance (using the angle transformation) showed that there was no significant difference between the chiasma frequencies of the proximal segments in *St/St* and *Sev/Sev* bivalents, and the same is obviously true for the distal segments. However, *St/Sev* bivalents have a significantly higher chiasma frequency in the distal segment than either of the homozygous types. It will be seen that in *St/Sev* bivalents the chiasma frequency of the proximal region (except in the Beaver individuals) is virtually zero. Thus we may say that chiasma formation is almost completely suppressed within the structurally heterozygous region. But heterozygosity for a proximal rearrangement seems to actually increase the chiasma frequency of the regions beyond the rearrangement (from approximately 1.10 to 1.37).

Any chiasmata formed in the "prohibited" proximal region of *St/Sev* bivalents would lead to chromatids carrying duplications and deficiencies (WHITE 1951). Such chiasmata, if they occurred, would be expected to cause partial sterility. In the Cherry Creek and Milford material only 3 such chiasmata (out of a total of 3033) were found. In the three individuals from Beaver the chiasma frequencies of the two regions of the heterozygous bivalent are quite different from those found in the Cherry Creek and Milford populations: there is a significant chiasma frequency in the proximal region between the two centromeres (0.07 as contrasted with <0.001 in the Cherry Creek and Milford heterozygotes) and the chiasma frequency in the distal region is much lower. Since these were the only individuals collected at Beaver, no homozygotes are available for comparison and the data on the three heterozygotes have accordingly been omitted from the comparison between the three types of bivalents. It is possible that the three individuals from Beaver are heterozygous for a rearrangement which is cytologically indistinguishable from Sevier but is in fact different.

The individuals from Beaver are interesting since they prove conclusively that chiasmata can be formed in the "prohibited" proximal region of bivalents heterozygous for pericentric rearrangements. Regardless of whether Sevier is a shift or an inversion, the 25 bivalents (out of 343) which formed chiasmata in the prohibited region would have produced 50 sperm chromosomes with duplications or deficiencies, out of a total of 1372, i.e. about 3.6%. Thus if heterozygosity for Sevier is maintained at a high level in the Beaver population it must either confer a heterotic advantage sufficient to compensate for this loss of fertility, or the loss in fertility must be in reality less than appears from the data.

The Humboldt rearrangement in Trimerotropis gracilis

At the south end of the Humboldt Range, Pershing Co., Nevada, in a sagebrush environment at approximately 6000 ft elevation there exists a population of *Trimerotropis gracilis* in which chromosomes 5, 6, 7, 8 and 9 are heteromorphic. The rearrangement in chromosome 6 only involves a minute region adjacent to the centromere, so that it is most difficult to identify. Chromosomes 7 and 8 are both trimorphic, i.e. the population contains two different metacentric types of each as well as the

TABLE 2

Chiasma frequency data on the Humboldt rearrangement in T. gracilis*

Type of bivalent	No. of individuals	0 prox. 1 dist.	0 prox. 2 dist.	1 prox. 0 dist.	1 prox. 1 dist.	Mean Prox.	Mean dist.	Total bivalents
St/St	19	554	14	338	298	0.501	0.745	1204
St/Ht	10	612	3	—	—	—	1.005	615

* Complete data on file at the GENETICS Editorial Office.

acrocentric one. Chromosome 5, on the other hand, is represented only by two easily distinguished types, one acrocentric and one with an almost median centromere. The former will be referred to as the Standard type, the rearrangement present in the latter being called Humboldt. The Standard 5th chromosome occurs in this population with a frequency of approximately 0.73, the frequency of Humboldt being about 0.27 (figures based on an analysis of 559 male individuals or 1118 chromosomes collected in 1949, 1950, 1951).

For the purposes of the present investigation the Humboldt rearrangement has one serious disadvantage, since bivalents homozygous for Humboldt are easily confused with bivalents 1, 2, 3 and 4 (all of which are invariably metacentric in this population, with the single exception of chromosome 4, in one individual, noted below). Thus it was only possible to compare the chiasma frequencies of the proximal and distal segments in *St/St* and *St/Ht* bivalents, those of the *Ht/Ht* bivalents being impossible to determine without serious errors.

The chiasma frequency data for 19 *St/St* and 10 *St/Ht* males are given in table 2. In the case of *St/St* bivalents there is hardly ever any difficulty in deciding whether a chiasma lies in the proximal or the distal section. As in the case of *T. sparsa*, there was a most striking heterogeneity in the data for *St/St* individuals. However, there can be no doubt that the mean chiasma frequency of the distal segment rises from about 0.74 in the *St/St* bivalent to slightly above 1.0 in the *St/Ht* one, the difference being highly significant.

Rearrangements in chromosome 7 of Trimerotropis gracilis

In a few instances, in the Trimerotropine grasshoppers, two different pericentric rearrangements have managed to establish themselves in the same chromosome, thus creating a 'triad' of gene sequences. This is so in the case of the 7th chromosome in the Humboldt Range population of *T. gracilis*, which is represented by an acrocentric type (Standard) a metacentric with markedly unequal arms (Oreana) and another metacentric with nearly equal arms (Rochester). The frequencies of these types during the years 1949 and 1950 were approximately: Standard—0.123; Oreana—0.563; Rochester—0.314 (based on an analysis of 446 individuals, i.e. 892 chromosomes).

In this case we have six types of bivalent to consider: *St/St*, *St/Or*, *St/Roch*, *Or/Roch*, *Or/Or*, and *Roch/Roch*. In the *Or/Roch* bivalent there are three regions in which chiasmata might, theoretically, be formed. We may call these the proximal, median and distal segments. In the *St/Roch* and *Roch/Roch* bivalents there is nothing

TABLE 3

Chiasma frequency data on the rearrangements in chromosome 7 of T. gracilis*

Type of bivalent	No. of individuals	1 prox.	1 med.	1 dist.	1 dist. 1 prox.	Total bivalents
St/St	3	51	8	55	13	127
St/Or	5	—	13	237	—	250
St/Roch	5	—	—	250	—	250
Or/Roch	5	—	—	235	—	235
Or/Or	5	97	135		18	250

* Complete data on file at the GENETICS Editorial Office.

TABLE 4

Chiasma frequency of Roch/Roch *homozygotes**

Number of individuals	1 chiasma	2 chiasmata in same limb	1 chiasma in each limb	Total bivalents
6	254	4	8	266

* Complete data on file at the GENETICS Editorial Office.

to mark the boundary between the proximal and median segments, and in the *St/Or* and *Or/Or* bivalents there is nothing to mark the boundary between the median and distal segments; while in the *St/St* bivalents all three segments are arbitrary.

Chiasma frequency data for five different types of bivalents are given in table 3. In the case of the *Roch/Roch* bivalents there is no detectable difference in length between the two arms (one of which corresponds to the proximal + median segments, the other to the distal one); the data for this type of bivalent are accordingly given separately in table 4.

The general picture which emerges from these data on the chiasma frequencies of the various segments in the different kinds of bivalents is shown diagrammatically in figure 3 (it being assumed that the chiasma frequency of the two arms of the *Roch/Roch* bivalent is the same). It will be clear that the chiasma frequency of the proximal segment is zero in all the heterozygous combinations, and that the median segment also has a zero chiasma frequency in the *St/Roch* and *Or/Roch* bivalents. The chiasma frequency of the distal segment, which is <0.6 in the homozygous bivalents, rises to about 0.95 in *St/Or* and to 1.0 in *Or/Roch* and *St/Roch*.

Out of 250 *Or/Or* bivalents (table 3) 97 have a single chiasma in the short arm, 135 have a single one in the long arm and 18 have chiasmata in both arms. This indicates a chiasma frequency of 0.46 in the short arm and 0.61 in the long one. If there were no interference across the centromere, one would expect that 70.2 of the 250 bivalents would show chiasmata in both arms and 52.6 would lack a chiasma altogether. The data thus provide evidence of strong interference between the two arms of the bivalent.

An unusual type of rearrangement in Trimerotropis gracilis

A rearrangement which has several interesting properties was found in the heterozygous condition in a single individual in the Humboldt Range population of *T.*

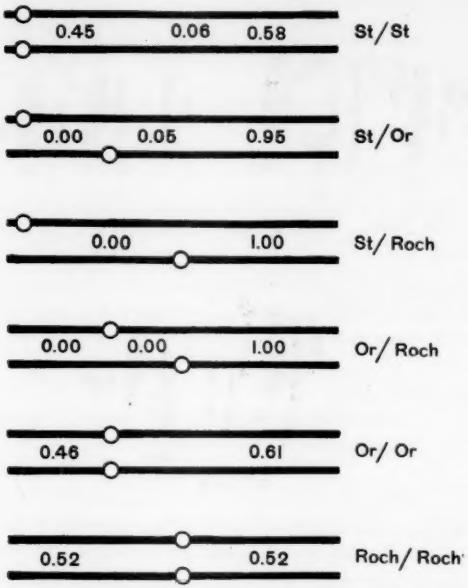


FIGURE 3.—Diagram showing the chiasma frequencies of the various chromosomal regions in the different kinds of 7th bivalents in the Humboldt Range population of *T. gracilis*.

gracilis (out of 559 collected in 1949–1951). In this individual, chromosome 4, which is invariably metacentric in the other 558 individuals, is represented by an acrocentric and a metacentric. It was possible to analyse 124 first metaphases in the testis of this grasshopper. In 23 of them there was a 4th chromosome bivalent of the usual heterozygous type (i.e. with a single chiasma in the distal segment); in 74 there was a chiasma in the 'short arm' of the acrocentric; and in the remaining 27 cells the 4th chromosome was represented by two univalents (fig. 4). In no case were chiasmata simultaneously present in the short arm and the distal segment, so as to produce a ring-bivalent.

Several tentative conclusions may be drawn from these data. Assuming that univalents segregate at random, about 11% of the gametes of the individual in question must have lacked chromosome 4 or have carried it in duplicate. Haploidy or trisomy for such a large element is likely to be lethal or strongly deleterious, and in fact no individuals carrying 1 or 3 4th chromosomes have been encountered. Thus, on the assumption that the aneuploid sperms are functional, the fertility of this individual was probably only about 89% of normal, which is much more serious than the loss of fertility in the three individuals of *T. sparsa* from Beaver (estimated at 3.5%). It is unlikely that any increase in viability, due to heterosis, will be great enough to compensate for a loss of fertility amounting to 11%. Thus the acrocentric type of chromosome 4 must be at a selective disadvantage in the Humboldt Range

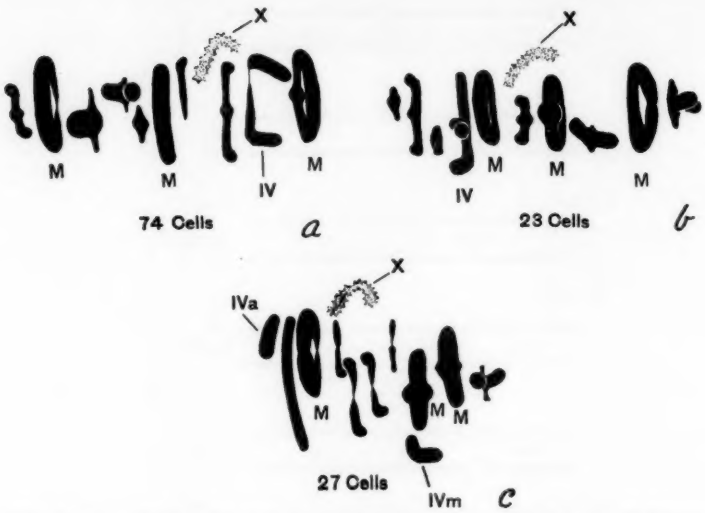


FIGURE 4.—Three types of first metaphases met with in an individual of *T. gracilis* heterozygous for an unusual type of rearrangement in chromosome 4.

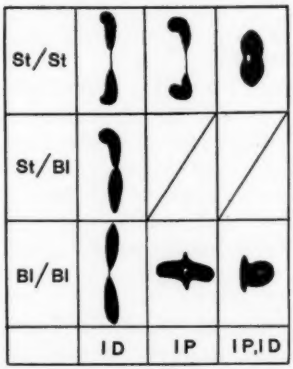


FIGURE 5.—The various forms assumed by the second largest chromosome in *Moraba scurra*, heterozygous and homozygous for the Standard and Blundell sequences, according to whether there is only a distal chiasma (1 D), only a proximal one (1 P) or a chiasma in both the distal and proximal regions (1 P, 1 D).

population. Since there is no evidence that it is transmitted to more gametes than the metacentric type, it could not be expected to persist at this locality. Its presence in a single individual may be due to its being a recent chromosomal mutation. More probably, it has reached the Humboldt Range locality by immigration from a colony

TABLE 5

Chiasma frequency data on the Blundell rearrangement in Moraba scurra†

Type of bivalent	No. of individuals	1 S.A.*	1 S.A. 1 dist.	0 prox. 1 dist.	1 prox. 0 dist.	1 prox. 1 dist.	Mean prox.	Mean dist.	Total bivalents
St/St	4	—	—	112	97	491	0.811	0.841	700
St/Bl	4	—	—	800	—	—	0.000	1.000	800
Bl/Bl	7	3	1	278	421	497	0.762	0.630	1200

* S.A.—a chiasma in the minute short arm of an acrocentric chromosome.

† Complete data on file at the GENETICS Editorial Office.

where it is not deleterious, either because the metacentric type of 4th chromosome is absent or in which, for one reason or another, univalents are not produced in 4th chromosome heterozygotes. The presence of a supernumerary chromosome in 2 out of the 559 individuals of the Humboldt Range population examined may be additional evidence in favor of the view that some immigration has occurred (a beneficial supernumerary should be much commoner and a deleterious one would be expected to become extinct unless its presence was due to immigration from a locality in which it was not deleterious).

The fact that the heterozygous 4th chromosome bivalents form chiasmata *either* in the short arm *or* in the external segment (but not in both) is remarkable. If chiasma-formation in these two segments were independent we should expect approximately 13.7 of the 124 bivalents to possess chiasmata in both segments, whereas in fact none of them did so. Formally, the situation might be ascribed to strong chiasma interference. But a type of interference which extends over a long pericentric rearrangement is unprecedented, and it seems more likely that the mechanism of synapsis in this bivalent is peculiar in some way and permits pairing in either the short arm or the external segment but not in both regions of the same bivalent.

The Blundell rearrangement in Moraba scurra

A description of the cytology of this species will be published elsewhere, as part of a general account of the cyto-taxonomy of the genus Moraba and its relatives. For the present it will be sufficient to state that *M. scurra* has 2n ♂ = 15 and that the Blundell rearrangement occurs in the second largest chromosome, which cannot be confused with any other member of the complement. There is some reason to believe that this element was originally metacentric and derived by fusion between two acrocentric chromosomes C and D which are separate in some other members of the genus. Thus we shall refer to the condition where both second chromosomes are acrocentric as *Bl/Bl*, individuals with two metacentric second chromosomes being regarded as homozygous Standard. The frequency of the metacentric (Standard) chromosome in the population studied is about 0.49, that of the Blundell chromosome being approximately 0.51 (based on a sample of 52 individuals, i.e. 104 chromosomes).

As shown in table 5 the chiasma frequency of the distal segment of the bivalent was always 1.0 in the *St/Bl* heterozygotes, but averaged 0.84 and 0.63, with a range from 0.22 to 0.97 in the homozygotes. Thus, in spite of considerable heterogeneity among both classes of homozygotes, there is a highly significant increase in the

chiasma frequency of the distal segment in the heterozygotes, exactly as in the species of Trimerotropis. The chiasma frequency data for the proximal and distal segments of the second bivalent in the *St/St* individuals, like those for the Oreana homozygotes of *T. gracilis*, provide evidence for interference across the centromere region.

Chiasma frequency and structural heterozygosity in a population of Trimerotropis suffusa

In the examples discussed above, the reduction in the chiasma frequency of the various bivalents, caused by the structural heterozygosity, depends largely on the homozygous chiasma frequency. Thus the reduction was 0.57 for the Sevier rearrangement in the largest chromosome of *T. sparsa*, in which the average chiasma frequency of homozygous bivalents was 1.94. In the heterozygous bivalents of *T. gracilis* (Humboldt rearrangement and the various sequences of chromosome 7) and in those of *M. scurra*, the chiasma frequency in all cases was 1.00, the reduction being 0.24, 0.07 and 0.52. There are, in addition, in the Trimerotropine grasshoppers, many instances of small chromosomes with pericentric rearrangements, whose chiasma frequency is always 1.0 (i.e. in which structural heterozygosity causes no change in the chiasma frequency of the chromosome as a whole, although it undoubtedly affects the chiasma frequency of the various regions). In all cases where the chiasma frequency of the homozygous bivalent is greater than 1.0, it seems likely that structural heterozygosity causes a diminution in chiasma frequency. This diminution must be small in some cases, but may be greater than 0.5 in bivalents with high chiasma frequencies.

We may now inquire what the situation is in populations with much cytological polymorphism, i.e. with many multiple heterozygotes. In particular, we wish to know whether the reduction in chiasma frequency which occurs in heterozygous bivalents is compensated for, to any extent, by a rise in the chiasma frequency of the other members of the chromosome set, i.e. whether the "Schultz-Redfield effect" operates in these grasshoppers. In an attempt to answer this question the chiasma frequencies of 45 individuals of *Trimerotropis suffusa* from a population inhabiting the vicinity of Truckee, California were determined. The number of structurally heterozygous bivalents in these individuals ranged from 0 to 6. In most cases the individual bivalents cannot be identified with certainty, so that only the total chiasma frequency of the chromosome complement could be ascertained (there are 11 autosomal bivalents in this species). In the absence of the Schultz-Redfield effect one would expect the total chiasma frequency to decline as the number of heterozygous bivalents increases. The extent of this decline would depend on the homozygous chiasma frequency of the bivalents which were heterozygous. Thus if all rearrangements were in bivalents whose homozygous chiasma frequency was 1.5 or greater, a decline in total chiasma frequency of at least 0.5 per heterozygous bivalent would be expected. However, the decline would be much less if rearrangements were above average frequency in "short" chromosomes. There is no reason for believing that in *T. suffusa* rearrangements are more frequent in chromosomes with few chiasmata per bivalent. In the Truckee population (excluding chromosome 10—see table 6) the average total chiasma frequency of the 28 individuals with 0, 1 and 2 heterozygous

TABLE 6

Chiasma frequency per nucleus in 45 individuals of Trimerotropis suffusa *from Truckee, California*

No. of bivalents heterozygous*	No. of individuals studied†	Mean chiasma frequency of 11 bivalents	S.E. of mean‡	M.S. (individuals)	M.S. (residual)
0	3	14.2	±0.56	7.0**	0.98
1	12	14.4	±0.28	24.9**	0.88
2	13	14.6	±0.26	33.2**	0.94
3	10	14.2	±0.30	5.0**	0.86
4	6	13.9	±0.39	4.8**	0.64
5	1	13.8	±0.98	—	0.68
Total	45	14.3	±0.14	19.4**	0.86

* The small 10th bivalent is disregarded in this column, since its chiasma frequency is invariably 1.0, regardless of whether it is homozygous or heterozygous. Thus some individuals counted as having x bivalents heterozygous actually had x + 1.

† 20 cells scored in each individual.

‡ Estimated as $\sqrt{\frac{I}{n} + \frac{R}{20n}}$ where I is the variance among individuals (estimated from the whole sample) and R is the variance among cells in individuals.

** Significant at 1 percent level.

bivalents is 13.47. Assuming that chiasma frequency and rearrangement frequency are independent, one would expect a decline in total chiasma frequency of approximately 0.35 per heterozygous bivalent if the Schultz-Redfield effect were not operating.

On the other hand, if the Schultz-Redfield effect were operative, one would expect no decline in total chiasma frequency until a stage which we may term "interference saturation" is reached (i.e. beyond a certain point one would expect that the Schultz-Redfield effect would be unable to compensate for the loss of chiasmata due to increasing heterozygosity). The data obtained are set out in table 6. Analysis of variance shows that the differences in chiasma frequency between the six classes of individuals are not significant. (Mean square among heterozygous classes only 0.6 as large as that among individuals in classes.) Taking the whole of the data, the regression of chiasma frequency on number of heterozygous bivalents is -0.13 ± 0.14. The negative sign of the regression is derived entirely from the 7 individuals in the last two classes (those with 4 and 5 heterozygous bivalents). It is possible that this is the level at which "interference saturation" is reached. Since we do not know just how much the chiasma frequency of the individual bivalents is decreased by structural heterozygosity, a rigorous proof that the Schultz-Redfield effect is operating is not possible. However, the data do very strongly suggest that it is operative, and in any case indicate that in a population with extreme structural hybridity there is little or no difference in chiasma frequency between the most heterozygous individuals and the structurally homozygous ones.

If we include chromosome 10, which is ignored in table 6, the mean heterozygosity of the Truckee population is 2.6 (i.e. each individual has, on the average, 2.6 structurally heterozygous bivalents). The eastern race of *T. suffusa*, which inhabits Colo-

TABLE 7

Comparison between the Truckee and Archuleta County populations of T. suffusa

	No. individuals	Mean chiasma frequency	S.E. Mean	M.S. (individuals)	M.S. (residual)
Truckee	45*	14.3	±0.14	18.5**	0.86
Archuleta Co.	10*	15.8	±0.25	12.3**	1.43
Difference		1.5**	±0.28		0.57**

* 20 cells analyzed in each individual.

** $P < 0.01$.

rado and Wyoming, is much less heterozygous, a population from Archuleta Co., Colorado having a mean heterozygosity of 0.3. As shown in table 7, the chiasma frequency of the individuals in this population is significantly higher than in the Truckee population. There is also a higher variance in the number of chiasmata per cell in the Archuleta Co. individuals.

The data in table 6 do not suggest that the difference between the chiasma frequencies of these populations is determined by differences in heterozygosity. The increased chiasma frequency of the Colorado population seems more likely to result from decreased interference. The difference in variance among cells of individuals may reasonably be ascribed to the same cause, especially as there is no significant or important difference among residual variances of the classes of table 6. The excess chiasma frequency, over the 11 necessary for normal disjunction, is 4.8 in the Colorado population and 3.3 in the Truckee population. The ratio of the excess chiasmata is 1.45 and of variances 1.66. These ratios agree well within the sampling errors of such estimates, suggesting that both increased chiasma frequency and increased variance among cells are expressions of decreased interference.

Differences in total chiasma frequency among individuals of the Truckee population may be caused in part by the chiasma frequencies of those bivalents which are heterozygous for a rearrangement. However heterogeneity among individuals without heterozygous bivalents in this and also in the Colorado population may well reflect genetic variations. Differences between the populations are presumably largely genetic in origin. Therefore there seem to be reasonable grounds for believing that the degree of interference has different optima in different localities and that this character is subject to natural selection.

DISCUSSION

In such organisms as grasshoppers the chromosomes are only held together in bivalents after pachytene by the presence of chiasmata. If a chiasma is not formed, the two chromosomes will fall asunder and behave as univalents which will pass to the same anaphase pole at the first meiotic division in approximately 50% of cases. Thus in grasshoppers the only rearrangements which can be expected to survive are those which leave the chromosome with a chiasma frequency $\geqslant 1.0$ in all heterozygous and homozygous combinations, and in all genotypes which it may encounter in the population. The rearrangements which we can study in natural populations

are hence not a random sample, natural selection having eliminated any which reduced the chiasma frequency to a point where regularity of disjunction was adversely affected. The situation may be different in Drosophila where, as COOPER (1945) puts it, chiasmata are only a sufficient and not a necessary cause for normal segregation.

It is accordingly not surprising to find that the pericentric rearrangements of grasshoppers, when heterozygous, do actually increase the chiasma frequency of the distal region (although, in the case of the Sevier rearrangement the increase seems to be greater than is strictly necessary to ensure regular disjunction). The actual mechanism responsible for producing this increase is almost certainly the ***removal of the interference*** which in structurally homozygous bivalents is caused by the presence of chiasmata in the proximal region. The physical basis of chiasma interference is, of course, still unknown, but the phenomenon itself is probably found in all species that form chiasmata at meiosis (HALDANE 1931; CALLAN 1949; CALLAN and MONTALENTI 1947; MONTALENTI 1947).

In general, we may regard chiasma interference as an adaptive property of genetic systems, since it permits each bivalent to form at least one chiasma, without the bivalents having an inordinately high chiasma frequency (if there were no chiasma interference and the numbers of chiasmata followed the Poisson distribution the mean would have to be over 5 per bivalent in order to ensure a negligible proportion of bivalents with 0 chiasmata). The existence of this interference has permitted certain pericentric rearrangements to establish themselves in grasshopper populations without causing frequent nondisjunction. But it was certainly present in the species long before the origin of the rearrangements.

The effects of structural rearrangements in grasshopper chromosomes on the chiasma frequency of the region outside the rearrangement appear at first sight very different from those found in the case of Drosophila by DOBZHANSKY and EPLING (1948) and other workers. There can be no doubt that this difference is partly genuine. To some extent, however, it may reflect the respective disadvantages of the genetic and cytologic methods of analysis. DOBZHANSKY and EPLING were only able to study crossovers in the genetically marked regions of the chromosome; it is at least conceivable that crossing over was actually increased beyond the marked region, as in the case of *D. virilis*, studied by KOMAI and TAKAKU. Conversely, in the present work, the data by no means exclude the possibility of a decrease in the chiasma frequency of the region immediately adjacent to the rearrangement—but if such a decrease exists it must be more than compensated for by an increased distad.

The data from the Truckee population of *T. suffusa* provide some evidence that the Schultz-Redfield effect is not confined to Drosophila. It is probable that the mechanism underlying the effect is a very general one, since MATHER and LAMM (1935) demonstrated that in Secale and Vicia the variation in chiasma frequency among cells was less than that which would be expected on the basis of the distribution of chiasmata in bivalents in cells (assuming in the case of Secale that the various bivalents have the same chiasma frequency). These results suggested that a reduction in chiasma frequency in one bivalent was compensated by increased chiasmata in one or more other bivalents. In other words, there was a tendency for individual cells to have very similar numbers of total chiasmata. Rearrangements are unlikely to

have been important in the material studied by MATHER and LAMM. Therefore a mechanism probably exists for damping variation in total cell chiasma frequency, whether such variation arises from structural heterozygosity or some other cause. (Using some recently popular terminology, one might state that the Schultz-Redfield effect is an expression of a "homeostatic" mechanism which maintains chiasma frequency close to some value which is presumably optimal for the population.)

SUMMARY

1. In three species of grasshoppers, pericentric rearrangements effectively suppress chiasma formation within the region heterozygous for the rearrangement.

2. The chiasma frequency of regions distal to the rearrangement is, however, increased by heterozygosity for pericentric rearrangements.

3. These results are interpreted in terms of chiasma-interference. In several instances it is shown that interference may extend across the centromere.

4. Presumably due to the operation of the "Schultz-Redfield" effect, in a population of grasshoppers containing from 0 to 6 structurally heterozygous bivalents per individual, the frequency of structural heterozygosity has little or no influence on total chiasma frequency.

ACKNOWLEDGMENTS

The material of *Trimerotropis sparsa*, *gracilis* and *suffusa* was collected with the aid of grants from the American Philosophical Society and the Research Institute of the University of Texas to the senior author. DR. T. C. HSU, MR. FREDERICK L. PETERSEN and MR. HOMER W. PHILLIPS helped to collect the material in the field. We are grateful to DR. MARVIN WASSERMAN, DR. FRANCES E. CLAYTON and MR. W. L. EVANS, formerly graduate students at the University of Texas, for assistance in the preparation of the slides.

LITERATURE CITED

CALLAN, H. G., 1949 Chiasma interference in diploid, tetraploid and interchange spermatocytes of the earwig, *Forficula auricularia*. J. Genet. **49:** 209–213.

CALLAN, H. G., and G. MONTALENTI, 1947 Chiasma interference in mosquitoes. J. Genet. **48:** 119–134.

CARSON, H. L., 1953 The effects of inversions on crossing over in *Drosophila robusta*. Genetics **38:** 168–186.

COOPER, K. W., 1945 Normal segregation without chiasmata in female *Drosophila melanogaster*. Genetics **30:** 472–484.

DOBZHANSKY, TH., and C. EPLING, 1948 The suppression of crossing over in inversion heterozygotes of *Drosophila pseudoobscura*. Proc. Nat. Acad. Sci. U. S. **34:** 137–141.

HALDANE, J. B. S., 1931 The cytological basis of genetical interference. Cytologia **3:** 54–65.

KOMAI, T., and T. TAKAKU, 1940 Two independent inversions in the X-chromosome of *D. virilis* and their effects on crossing over and disjunction. Cytologia **11:** 245–260.

1942 On the effect of the X-chromosome inversions on crossing over in *Drosophila virilis*. Cytologia **12:** 357–365.

MATHER, K., and R. LAMM, 1935 The negative correlation of chiasma frequencies. Hereditas **20:** 65–70.

MONTALENTI, G., 1947 L'assetto cromosomico e l'interferenza dei chiasmi nella spermatogenesi di *Simulium equinum* L. (Diptera, Simuliidae). R.C. Accad. Lincei (8) **2:** 471–474.

MORGAN, T. H., C. B. BRIDGES, and J. SCHULTZ, 1930 The constitution of the germinal material in relation to heredity. Carnegie Inst. Wash. Ybk. **29:** 352–359.

SCHULTZ, J., and H. REDFIELD, 1951 Interchromosomal effects on crossing over in Drosophila. Cold Spring Harbor Symposia Quant. Biol. **16:** 175–197.

STEINBERG, A. G., 1936 The effect of autosomal inversions on crossing over in the X-chromosome of *Drosophila melanogaster*. Genetics **21:** 615–624.

1937 Relations between chromosome size and effects of inversions on crossing over in *Drosophila melanogaster*. Proc. Nat. Acad. Sci. U. S. **23:** 54–56.

STEINBERG, A. G., and F. C. FRASER, 1944 Studies on the effects of X-chromosome inversions on crossing over in the third chromosome of *Drosophila melanogaster*. Genetics **29:** 83–103.

STURTEVANT, A. H., and G. W. BEADLE, 1936 The relations of inversions in the X-chromosome of *Drosophila melanogaster* to crossing over and disjunction. Genetics **21:** 554–604.

WHITE, M. J. D., 1951 Structural heterozygosity in natural populations of the grasshopper *Trimerotropis sparsa*. Evolution **5:** 376–394.

1954a *Animal Cytology and Evolution*. Second edition. Cambridge University Press.

1954b An extreme form of chiasma localization in a species of Bryodema (Orthoptera, Acrididae). Evolution **8:** 350–358.

BACKCROSS STUDIES ON THE GENETICS OF RESISTANCE TO MALARIA IN MICE[1]

ELI M. NADEL,[2] JOSEPH GREENBERG,[3] GEORGE E. JAY,[4] AND G. ROBERT COATNEY,[3]

National Institutes of Health, Bethesda 14, Maryland[5]

Received January 14, 1955

IT IS well known that studies on experimental malaria in animals have yielded valuable information on the applied chemotherapy of this disease in man. Less appreciated is the fact that the Plasmodium organism can be a valuable research tool in fundamental studies no less important than work on other microorganisms. Use of *Plasmodium berghei* and *Plasmodium gallinaceum* in this sense has yielded basic information and has stimulated interest in (1) the mode of action of drugs in infections and parasitic diseases other than malaria, (2) the mechanisms of drug resistance and the relationship between molecular structure and activity, (3) the interrelationships between the metabolism of parasitic and infectious diseases and cancer, (4) the endocrine aspects of erythropoieses, (5) the nature of the physiologic alteration following X-radiation, (6) the genetics of the invading organism, and (7) the genetics of host resistance. The following report concerns itself with survival of mice after malarial infection, and suggests that in this species resistance is probably largely genetically controlled.

Differences in the duration of survival after experimental infection with *Plasmodium berghei* have been demonstrated in certain inbred strains of mice and their F_1 hybrids. In 48 series utilizing 496 mice, C57 BL/6 and C57 L animals survived significantly longer than all other strains tested. In 38 series utilizing 385 mice, F_1 hybrids of C57 BL/6 or of C57 L mice as one parent, with one exception, survived significantly longer than other tested strains or hybrids. The solitary exception was the mutual F_1 hybrid, C57 BL/6 × C57 L F_1, which was less resistant than either parent. These results, summarized below (table 1), suggested that multiple genetic factors were probably effecting resistance to malaria. Additional data now reported on other hybrids, on backcrosses of the (C57 BL/6 × DBA/2) F_1 hybrid to either parent, and on the F_2 generation, collected concurrently with the previously reported studies, support the thesis for the genetic basis of resistance to malaria in mice. (GREENBERG, NADEL, and COATNEY 1953 and 1954; NADEL, GREENBERG, and COATNEY 1954; NADEL, GREENBERG, JAY, and COATNEY 1954).

METHODS

All animals were bred and weaned at the Animal Production Section, Laboratory Aids Branch of the National Institutes of Health; the subscripts JN have been

[1] Part of this material appeared as an abstract in the Proceedings of the 51st annual meeting of the American Society of Pathologists and Bacteriologists in April 8–10, 1954, in Philadelphia, Pa.

[2] Laboratory of Pathology, National Cancer Institute.

[3] Laboratory of Tropical Diseases, National Microbiological Institute.

[4] Laboratory Aids Branch, National Institutes of Health.

[5] Public Health Service, Department of Health, Education, and Welfare.

TABLE 1

Duration of survival after experimental infection with Plasmodium berghei

Group	Number of animals	Mean survival (days)	Standard error	Variance
Backcross and F_2 animals				
1 DBA/2*	70	9.9	0.46	14.82
2 C57 BL/6*	51	17.6	0.64	20.88
3 (C57 BL/6 × DBA/2)F_1*	44	21.2	0.45	8.88
4 (C57 BL/6 × DBA/2)F_2	174	17.1	0.54	49.84
5 (C57 BL/6 × DBA/2)F_1 × DBA/2	169	13.8	0.48	38.94
6 (C57 BL/6 × DBA/2)F_1 × C57 BL/6	81	20.0	0.93	70.05
F_1 hybrids				
7 (STR/N × A/LN)F_1	165	18.2	0.58	
8 (STR/N × C57 BL/6)F_1	103	24.5	0.44	
9 (STR/N × DBA/2)F_1	135	17.9	0.47	
10 (C57 L × DBA/2)F_1	140	19.3	0.50	

Statistical evaluation for significance ($P < 0.01$) showed that; Groups 1 and 5 are different from all other groups and each other; group 2 is different from 1, 3, and 6; group 3 is different from 1, 2, 4, and 5; there is no difference between groups 2 and 4 or between 3 and 6; group 8 was significantly different from groups 2, 4, 7, 9 and 10; there was no difference amongst groups 7, 9 and 10.

* Data previously reported but presented for comparative purposes (GREENBERG, NADEL, and COATNEY 1953 and 1954).

For comparative purposes, the following is a summary of survival data on all inbred strains and hybrids previously reported (GREENBERG, NADEL, and COATNEY 1953 and 1954):

F_1 hybrids: (C57 BL/6 × C57 L) (11.3); (BALB/c × A/LN) (13.2); (C57 BL/6 × C57 BR/cd) (15.1); (A/LN × DBA/2) (15.1); (BALB/c × C_3H/He) (15.1); (BALB/c × DBA/2) (16.4). (C57 L × A/LN) (19.3); (C57 BL/6 × A/LN) (22.7).

Inbred strains: A/LN (8.4); SWR (8.8); C_3H/He (9.7); C58/LN (10.4); STR/N (10.5); BRSUNT/N (10.7); C57 BR/cd (12.3); BALB/c (12.5); RIL (AKR/Lw) (14.4); C57 L (15.0).

deleted from the nomenclature used in the test. For the sake of brevity (C57 BL/6 × DBA/2) F_1 animals are referred to as BDF_1, while the F_2 hybrids of this mating (C57 BL/6 × DBA/2) × (C57 BL/6 × DBA/2) are referred to as BDF_2.

Survival studies on 3 groups of animals are reported. These include backcross studies on BDF_1 × C57 BL/6, and BDF_1 × DBA/2 and reciprocal matings, studies on the BDF_2 and reciprocals, and studies on the F_1 hybrids of female STR/N and male A/LN, C57 BL/6 or DBA/2 mice, as well as the C57 L × DBA/2 F_1 hybrid.

The Kasapa strain of *Plasmodium berghei* was used. The strain was maintained by weekly passage of infected blood through non-inbred Swiss mice. The Swiss mice served as donors of infected blood for all the experimental mice. Heparinized infected heart blood was diluted with physiological saline and each mouse received through a tail vein approximately 1,000,000 parasitized erythrocytes, in 0.1 ml of inoculum. Blood smears were examined on the third or fourth day after inoculation to establish the presence of the infection.

Mice of both sexes and approximately two months old (plus or minus 1 week) were used. Four to eight groups of mice infected on the same day with the

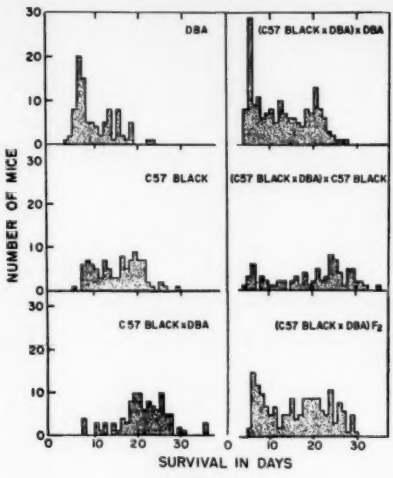


FIG. 1

same inoculum formed a single series, and in each series 5 Swiss mice were included. Each hybrid or backcross was represented by 4 to 24 individuals in from 4 to 13 series.

RESULTS

All of the animals died with malaria between the 5th and 36th day after inoculation. In all groups there were deaths as early as the 5th or 6th day.

Group I. Backcross study. Offspring resulting from the resistant parent strain (C57 BL/6, 17.60 ± 0.64 days) and its more resistant hybrid (BDF_1, 21.23 ± 0.45 days) lived longer than offspring of the same hybrid and its less resistant parent (DBA/2, 9.90 ± 0.46 days). This difference in survival, 20.0 ± 0.93 days for BDF_1 × C57 BL/6 mice versus 13.8 ± 0.48 days for BDF_1 × DBA/2 mice is significant (fig. 1 and table 1).

Group II. F_2 group. No significant difference was detected between the survival of BDF_2 or DBF_2 animals. The overall average survival of the F_2 group was 17.1 ± 0.54 days. (fig. 1 and table 1).

Group III. New hybrids. Heterosis was noted in each of the four previously untested F_1 hybrids as seen in table 1. The longest lived F_1 hybrid, (STR/N × C57 BL/6) F_1, lived 24.5 ± 0.44 days after experimental injection with *Plasmodium berghei*. No significant difference was noted in the survival of (STR/N × A/LN)F_1 hybrids (18.2 ± 0.58) in comparison with (STR/N × DBA/2) F_1 hybrids (17.9 ± 0.47).

DISCUSSION

Additional evidence has been presented to support the thesis of a genetic basis of resistance to malaria in mice. Including this report, we have now studied survival after infection with *Plasmodium berghei* in over 2000 mice from 12 inbred strains and

from 13 different F_1 hybrid crosses of these strains, as well as the backcrosses and F_2 hybrids from one of the more resistant hybrids (BDF_1). The 12 inbred strains have been found to fall into a short, intermediate, or a long survival group, with further subdivision probably present in the intermediate group. Of the 13 different kinds of F_1 hybrids tested, 10 have exhibited significant heterosis in respect to resistance to malaria; two hybrids, (BALB/c × A/LN) F_1 and (C57 BL/6 × C57 BR/cd) F_1, showed no significant heterosis but proved more resistant than one of their parents; and one hybrid (C57 BL/6 × C57 L) F_1 was significantly less vigorous than either parent. It has been suggested that in classical studies on inbred corn, increased vigor (heterosis) in a hybrid is to be expected where many different genes are involved, while decreased vigor occurs in F_1 hybrids resulting from the mating of closely related parents (GOWEN 1952). The analogy between increased vigor in corn and increased resistance to malaria is reinforced by the decrease in resistance observed in the (C57 BL/6 × C57 L) F_1 animals, hybrids of closely related parent strains. Further studies on closely related strains and their hybrids would be helpful in deciding whether a smaller number of gene differences in the parents makes for decreased resistance to malaria in the F_1 hybrid as is implied in our results.

The results obtained with the BDF_1 and BDF_2 hybrids of C57 BL/6 and DBA/2 mice, and with the backcrosses of the BDF_1 to each of the parents, would seem to support the genetic basis of resistance to malaria. The BDF_1 hybrids exhibited heterosis, while the BDF_2 exhibited none. The latter survived no longer than the longer-lived parent (fig. 1 and table 1). The backcross of the BDF_1 to DBA/2 was significantly shorter-lived than the C57 BL/6 parent, the BDF_1, the BDF_2 hybrids, and the backcrosses to the C57 BL/6 parent, but was however, longer-lived than the DBA/2 parents. The backcross to C57 BL/6 was longer-lived than the C57 BL/6 parents and behaved very much like the BDF_1 hybrids. Since the maternal parents in both backcrosses were the same, one can safely eliminate ordinary maternal influences as a major factor in the differences in survival of the backcross mice. In addition, in our studies, coat color appeared to be of no significance in the survival of mice of either the backcrosses or BDF_2 hybrids.

The wide range of survival time in all groups studied, and the lack of good correlation between individual extreme survival time within a group, with the mean survival of the group, prevent a clear-cut genetic analysis of the data.[6]

When all our survival data were combined, deaths appeared to have occurred in 2 distinct waves with one peak on the 6th and the other on the 21st day after inoculation (figure 2). Referring back to our individual strain and hybrid data, it appeared that most short survival mice, (e.g., A/LN, DBA/2) died in the first wave of deaths while almost all of the long survival mice (e.g., (STR/N × C57 BL/6) F_1 hybrids) died in the second wave. With increase in survival time there appeared to be a shift in deaths from the first to the second wave. However, even in the longest-lived hybrids, a small proportion of the mice died early. While this is probably an oversimplification, it might be said that the mean survival data we have been collecting

[6] DR. JOHN W. GOWEN, Professor of Genetics, Iowa State College, helpfully pointed out that the possibility of a one-gene-difference with a sizable amount of variation due to environmental circumstances should also be considered.

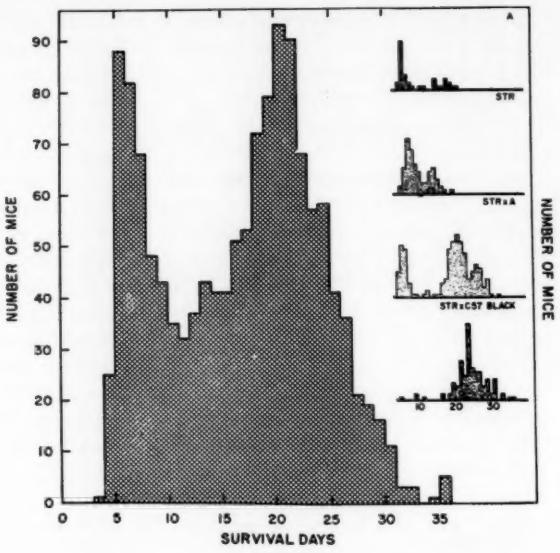


FIG. 2

are an expression of the proportion of the mice which survive the early wave of deaths.

The wide range of death observed in all series suggests that extragenetical as well as genetical factors may be implicated in death from malaria in mice. Nonetheless the consistent reproducibility of the mean survival data for each strain and hybrid is striking and significant. The same is true of the backcross studies. If extragenetical factors are also involved, their superimposition on a stable gene background may still yield complicated but reproducible patterns for each inbred strain and hybrid. In addition to the strain differences observed, the evidence for a genetic basis for increased survival resides in the demonstration of (1) heterosis, (2) significantly increased survival in the backcrosses to the more resistant parent, and (3) increased variance in the F_2 and backcross animals in comparison to that of the parent strains or the F_1 hybrids (table 1).

Although survival time after experimental infection was the criterion applied in classifying strains for their resistance to malaria, other measurements may prove to be of additional value in further defining the nature of the strain differences. We found that survival reflected in the simplest measurable and reproducible manner the overall ability of a strain to resist the physiological insults of malarial parasitization. Indeed, without recorded survival data, no knowledge of strain differences would have been apparent. *Total parasitemia* itself gave *no* inkling that significant strain differences existed. In our earlier study on DBA/2, C57 BL/6 and their BDF_1 hybrids, average survival was 9.9, 17.6 and 21.2 days, respectively, yet the rate of increase of

total parasitemia was approximately the same for all three groups (GREENBERG, NADEL, and COATNEY 1953). However, the diphasic nature of all combined survival data awakened new interest in the slight notch observed in the earlier reported total parasitemia curve for the BDF_1 hybrids, and suggested that attention be given to a more detailed parasitemia analysis. A preliminary study has indicated that early deaths are associated with a high degree of infection of mature erythrocytes, while late deaths were associated with a high degree of infection of young erythrocytes, coupled with a gradual increase in the proportion of young erythrocytes in the total erythrocyte population. In most of the longer lived animals, the infection of mature erythrocytes was checked when a relatively small percentage of them had been infected. However, the correlation between survival and maximal degree of invasion of mature erythrocytes may not, from these preliminary studies, be a completely direct one. In eight strains studied, the order of decreasing parasitemia of mature erythrocytes on the sixth day after experimental infection has been found to be (1) non-inbred Swiss (40%), (2) DBA/2 (33%), (3) C_3H (25%), (4) C57 BL/6 (17%), (5) A/LN (15%), (6) BALB/C (13%), (7) STR (7%), and (8) BRSUNT (2%). This does not directly correlate with the order of increasing survival presented in the footnote in table 1. Survival-time though a reflection of presumably many factors, is a better reflection of overall host resistance.

SUMMARY

A comparison was made of the differences in the duration of survival after experimental infection with *Plasmodium berghei* in 12 inbred strains of mice and in F_1 hybrids involving 13 combinations of these strains. In addition, the survival of backcross mice of the (C57 BL/6 × DBA/2) F_1 hybrid was compared to that of their short-survival (DBA/2) and their long-survival (C57 BL/6) parents, as well as to the (C57 BL/6 × DBA/2) F_2 hybrids. Data based on over 2000 mice indicate that hybrids of C57 BL/6 or of C57 L parents exhibited the longest mean survival time. With the exception of the (C57 BL/6 × C57 L) F_1 hybrid, and the (C57 BL/6 × C57 BR/cd) F_1 hybrid, all other hybrids of these parents exhibited heterosis in respect to increased survival after malaria infection. The presence of (1) heterosis, (2) greater variance in the F_2 and in the backcross animals in comparison to the variance in the parents and F_1 hybrids, and (3) the significant increased survival of the backcross to the long-survival parent in comparison to that of the backcross to the short-survival parent indicate that multiple genetic factors may be involved. However, although multiple genes may influence the differences in survival, extra-genetic factors are probably also present. The presence of, as yet undetermined, non-genetic factors is indicated by (1) the wide range in the time of death exhibited by mice in all series from short or long survival mice, and (2) the presence of a bimodal curve of death based on a compilation of the survival data of all mice tested without regard to strain background. Subsequent re-evaluation of the data on the individual strains and hybrids indicated that the peak of deaths in the short-survival mice coincided with the early peak on the overall bimodal curve, while the peak of death in the long-survival mice coincided with that of the late peak in the overall bimodal curve.

LITERATURE CITED

GREENBERG, J., E. M. NADEL, and G. R. COATNEY, 1953 The influence of strain, sex and age of mice on infection with *Plasmodium berghei*. J. Infectious Diseases **93:** 96–100.

GREENBERG, J., E. M. NADEL, and G. R. COATNEY, 1954 Differences in survival of several inbred strains of mice and their hybrids infected with *Plasmodium berghei*. J. Infectious Diseases **95:** 114–116.

GOWEN, J. W., 1952 *Heterosis*. Iowa State College Press, Ames, Iowa.

NADEL, E. M., J. GREENBERG, and G. R. COATNEY, 1954 The effect of malaria (*Plasmodium berghei*) on leukemia L_{1210} in mice. J. Infectious Diseases **95:** 109–113.

NADEL, E. M., J. GREENBERG, G. E. JAY, and G. R. COATNEY, 1954 Increased resistance to malaria in certain inbred mice, their hybrids and backcrosses. Am. J. Pathol. **30:** 658–659.

LINKAGE RELATIONS OF THE GENES HISTOCOMPATIBILITY-2 AND FUSED TAIL, BRACHYURY AND KINKY TAIL IN THE MOUSE, AS DETERMINED BY TUMOR TRANSPLANTATION[1]

SALLY LYMAN ALLEN[2]

Department of Zoology, University of Chicago, Chicago, Ill., and Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Me.

Received January 27, 1955

THE literature on the genetic and antigenic basis of tumor transplantation has been reviewed by BITTNER (1935), LITTLE (1941; 1947), HAUSCHKA (1952) and SNELL (1953b, c). In mice, the genetic loci involved were named "histocompatibility" loci by SNELL (1948), the prefix "histo" being used to denote the probable similarity of genes determining reactions to neoplastic and normal tissue transplants. One of these histocompatibility loci, *H-2*, was shown to be closely linked to the *Fu* locus by GORER, LYMAN and SNELL (1948). *H-2* was shown to be identical with that gene determining antigen II present in A strain erythrocytes, which GORER had previously demonstrated (1936, 1937).

In addition to *H-2* and fused tail (*Fu*), one or two other loci have been identified on the ninth chromosome in mice: kinky tail (*Ki* or Fu^{Ki}) and brachyury (*T*). Fused tail is a dominant mutation, viable in the homozygous condition on certain genetic backgrounds, and characterized by the production of kinks and twists in the tail. Penetrance of *Fu* in heterozygous animals is incomplete with "normal overlaps" being found, the frequency of which varies with the genetic background and sex of the fused parent. The frequency of overlapping is greater when the female introduces the *Fu* gene than when the male introduces it. Kinky tail (*Ki* or Fu^{Ki}) is phenotypically similar to fused tail and may be an allele at the *Fu* locus (DUNN and GLUECKSOHN-WAELSCH 1954). Unlike fused, the homozygote is never viable, and there does not appear to be a sex difference in production of normal overlaps, but genetic background may considerably alter its expression. Hereafter, the gene symbol *Ki* will be used in referring to kinky. Brachyury (*T*) is a dominant mutation, lethal in homozygous condition. Typically, it produces a shortened tail but is highly variable in expression depending upon genetic background. It may produce kinks or, on certain genetic backgrounds, tailless animals, or, on still other backgrounds, normal overlaps (ALLEN and SNELL unpublished). For additional details or the original references pertaining to the tail mutants described, see GRÜNEBERG (1952).

Fu, *Ki* and *T* are closely linked loci contained within a chromosomal segment of approximately 8 units (DUNN and CASPARI 1945). The order of the loci was uncertain but their evidence pointed to the most probable order as either (1) *Ki T Fu*

[1] This investigation has been aided by a John M. Prather research fellowship from the University of Chicago and by a two year, three month predoctoral fellowship from the National Cancer Institute. Inbred mice and animal cages were provided by the Roscoe B. Jackson Memorial Laboratory with the financial assistance of a grant-in-aid (EG-14) from the American Cancer Society on recommendation of the Committee on Growth of the National Research Council.

[2] Present address: Department of Zoology, University of Michigan, Ann Arbor, Mich.

TABLE 1

Linkage experiments involving the ninth chromosome

Authors	Loci tested	Sex	Crossover percent		Number of mice
			Halfvalue†	Total	
DUNN & CASPARI (1945)	*Fu-T*	♂	2.1	4.3	657
" "		♀	0	0	137
DUNN & CASPARI (1945)	*Ki-T*	♂	1.2	2.5	1128
" "		♀	3.0	5.9	542
" "		♂	2.3	4.6	395
DUNN & GLUECKSOHN-WAELSCH (1953)		♂, ♀		3–5	73+
DUNN & CASPARI (1945)	*Fu-Ki*	♂	0	0	209
" "		♀	1.7	3.4	296
DUNN & GLUECKSOHN-WAELSCH (1954)		♂	0	0	892
" "		♀	0	0	79
SNELL (1952)	*H-2-Ki*	♂, ♀	no tested crossovers		
SNELL, SMITH & GABRIELSON (1953) & ALLEN (unpublished)		♂, ♀		4.0*	75
SNELL (1952)	*H-2-Fu*	♂, ♀		5.6	19
SNELL, SMITH & GABRIELSON (1953) & ALLEN (unpublished)		♂, ♀		8.3*	707
ALLEN (1955)		♂		4.1*	268
"		♀		6.9*	73
SNELL (1952)	*H-2-T*	♂		4.1	73
"		♀		12.5	8
SNELL, SMITH & GABRIELSON (1953) & ALLEN (unpublished)		♂, ♀		14.3*	394

* Crossover percent based only on animals with tail abnormality; crossovers not tested genetically.

† Frequency of one crossover class.

or (2) *Fu Ki T*. To account for the recombinants from a cross of *Ki T*/*Fu* × normal, DUNN and CASPARI (1945) concluded that *Fu* does not lie between *Ki* and *T*. The linkage relations of these loci, one to another and to *H-2*, are summarized in table 1, where a listing is made of the crossover values obtained by various workers for the 6 chromosome intervals.

By making use of the linkage of *H-2* with the genes *Fu*, *Ki* and *T* it has been possible to establish 9 alleles and 2 subgroup alleles at the *H-2* locus and to determine their distribution in 21 inbred strains and 7 isogenic lines of the A strain (SNELL 1951a, 1951b; SNELL and HIGGINS 1951; SNELL *et al.* 1952; SNELL, SMITH and GABRIELSON 1953; SNELL unpublished; ALLEN 1955; HOECKER, COUNCE and SMITH 1954). Six alleles have so far been found in only one strain or stock each ($H\text{-}2^{dk}$ or $H\text{-}2^{a}$, $H\text{-}2^{f}$, $H\text{-}2^{p}$, $H\text{-}2^{q}$, $H\text{-}2^{r}$, and $H\text{-}2^{s}$), while 3 alleles are more commonly distributed ($H\text{-}2^{b}$, $H\text{-}2^{d}$ and $H\text{-}2^{k}$). The 2 subgroup alleles are $H\text{-}2^{d'}$ and $H\text{-}2^{b'}$. Strains

characterized by these alleles originally were classed as *H-2*^{d} and *H-2*^{k} but further genetic tests, on the basis of reaction to transplantation, indicated that the antigens produced by these alleles were slightly different in nature. Each of the alleles of *H-2* exerts an effect in the heterozygote as in the case with alleles determining the M-N blood groups in man.

Originally the type allele characteristic of the A strain was designated simply *H-2*. Subsequently, however, extensive genetic crosses revealed that animals of the heterozygous genotype *H-2*^{d}/*H-2*^{k}, irrespective of the source of these alleles, grow strain A tumors, thus reacting as if *H-2* = *H-2*^{d}/*H-2*^{k} (SNELL 1951b; SNELL, SMITH and GABRIELSON 1953). Mice bearing other *H-2* alleles in various combinations do not grow strain A tumors. Thus, the type allele belonging to the A strain is now redesignated *H-2*^{dk}, indicating the presence of two components responsible perhaps for producing two antigens or two antigenic factors. Eight antigenic factors have recently been demonstrated by a combination of tumor transplantation and red cell agglutination techniques (GORER and MIKULSKA 1954; HOECKER, COUNCE and SMITH 1954). Some of these factors are uniquely produced by one allele; others are common to several alleles. Under the scheme of these authors, *H-2*^{dk} = *H-2*^{a} and produces antigenic factors (C)DEK. *H-2*^{d} produces CD, *H-2*^{k}—CEK, and *H-2*^{b}—BE. Whether a single locus with components or two or more closely linked loci are involved in the production of these antigenic factors has not been determined.

MATERIALS AND METHODS

The *inbred strains* used were: A (*H-2*^{dk}/*H-2*^{dk}); BALB/c (*H-2*^{d}/*H-2*^{d}); AKR (*H-2*^{k}/*H-2*^{k}); C57BL/6 and C57BL/10 (*H-2*^{b}/*H-2*^{b}).

The *stocks* used were: A-*H-2*^{k}*Ki* (*H-2*^{k}*Ki*/*H-2*^{dk}+); A-*H-2*^{f}*Fu* (*H-2*^{f}*Fu*/*H-2*^{dk}+); A-*H-2*^{dk}*T* (*H-2*^{dk}*T*/*H-2*^{dk}+). These stocks are isogenic lines of the A strain produced by repeated backcrossing of animals heterozygous for *Ki*, *Fu* or *T* to the A strain for 7 or more generations so that more than 99 percent of their genes are derived from the A strain with the exception of that chromosomal segment containing the locus *Ki*, *Fu* or *T*.

Transplantable tumor 15091a, native to the A strain, was used throughout these experiments. Originally diagnosed as a medullary adenocarcinoma of the mammary gland by CLOUDMAN (1932), it was transplanted for the first time on December 11, 1928. A recent report indicates that it is a sub-tetraploid tumor as determined by chromosome counts and by the desoxyribose nucleic acid phosphorus content per cell (HAUSCHKA and LEVAN 1953; LEVAN and HAUSCHKA 1953). Genetically it usually gives one factor ratios, *H-2* being the locus involved. It does grow in a certain percentage of some foreign strains, although there appears to be a relationship between growth and the allele of *H-2* carried by the particular strain (SNELL *et al.* 1953). In crosses it acts as a "good" genetic tumor (SNELL, SMITH and GABRIELSON 1953).

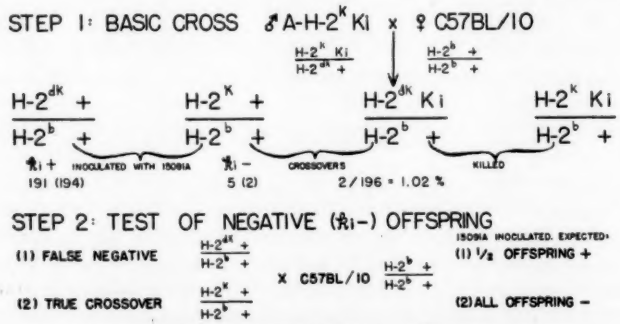
15091a was originally carried in the donor A strain by routine trocar inoculation. Because of difficulties encountered by infection, a more sterile technique had to be found. Therefore, for inoculating donor animals, the cytosieve, described by SNELL (1953a), was adopted. Experimental transplants, however, were always performed

by trocar. All animals receiving experimental transplants were checked weekly for tumor growth, starting 1 week after the tumor had been inoculated. The size of the tumor was recorded in terms of approximate square centimeters as viewed in two dimensions; this procedure was followed in order to eliminate from the data any animals dying from causes other than tumor growth. Animals were regarded as positive if they died from a progressively growing tumor. Death usually occurred from 3 to 6 weeks after the tumor was inoculated. Animals were regarded as negative if they never bore a palpable tumor or survived a small tumor which grew and then regressed.

CROSSOVERS BETWEEN H-2 AND KI

Since crossing over between *H-2* and *Ki* had not been adequately tested, crosses were set up to test for recombination. Figure 1 summarizes the crosses made and the results obtained. In the basis cross A-*H-2*^{k}*Ki* males (genotypically tested as *H-2*^{k}*Ki*/*H-2*^{dk}+) were mated to C57BL/10 females, and their normal-tailed offspring were inoculated by trocar at 6 to 8 weeks of age with A-strain tumor 15091a.

Of the normal-tailed offspring tested, the noncrossover class would be expected to succumb to 15091a while the crossover class would survive and could be further tested. Additional testing would not have been necessary if it were not for the presence of "false negatives" (see discussion in SNELL, SMITH and GABRIELSON 1953), animals genotypically positive but phenotypically negative to a transplantable tumor. Thus, a second cross was made in order to distinguish between "false negatives" and true crossovers. Resistant animals were crossed to the C57BL/10



RESULTS OF TESTING POSSIBLE CROSSOVERS

#	MATING	OFFSPRING: 15091A INOCULATED ++	+−	t	P	CONCLUSIONS
♂ 88	C57BL/10	18	26	1.205	.30	GENETICALLY +
	BALB/c	19	16	0.507	.70	
♀ 90	C57BL/10	0	39	6.245	<.001	CROSSOVER
♀ 91	C57BL/10	28	31	0.391	.70	GENETICALLY +
♂ 500	C57BL/10	3	8	1.508	.20	GENETICALLY +
♀ 501	C57BL/10	2	38	5.692	<.001	CROSSOVER

FIGURE 1.—Tests for crossovers between *H-2* and *Ki* (or *Fu*^{ki}).

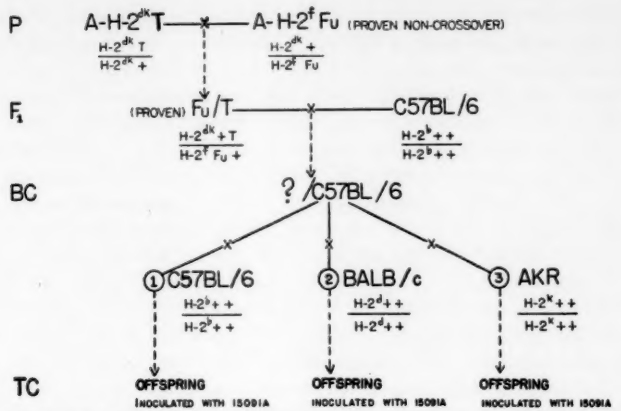


FIGURE 2.—Plan of matings for establishing the order of the loci *Fu*, *T* and *H-2*. BC = backcross generation; TC = testcross generation. For the genotype of F_1 *Fu/T* it should be noted that only one of the three possible orders of the loci is represented.

strain and inoculated with 15091a. Half the offspring from "false negatives" would be expected to grow 15091a, whereas all offspring from true crossovers should fail to grow 15091a.

In the first cross a total of 196 normal-tailed offspring were inoculated with 15091a. There were 5 survivors. From the second cross, it appeared that 3 of these animals were "false negatives" (♂88, ♀91, ♂500) and 2 were true crossovers (♀90, ♀501). A statistical test was run on these potential crossovers by comparing the ratios obtained with those expected for "false negatives," where:

$$t = \frac{\text{Observed value} - .5}{\text{S. E. } (q_o - q_T)}$$

Such testing revealed that the above supposition was correct, i.e. that only 2 of the 5 survivors were true crossovers. Therefore, it may be concluded that crossing over between *H-2* and *Ki* does occur, but with a low frequency. Where the heterozygous parent is the male, the indicated incidence is 1.02 percent (2/196). There are no data for crossing over in the heterozygous female.

ORDER OF THE LOCI FU, T AND H-2

Plan of the crosses

The plan of matings made is represented in figure 2. After appropriate matings to test for possible crossovers, mice of proven genotype $H\text{-}2^{dk}+/H\text{-}2^{f}Fu$ of the A-$H\text{-}2^{f}Fu$ stock were crossed to animals from the A-$H\text{-}2^{dk}T$ stock (genotype $H\text{-}2^{dk}T/H\text{-}2^{dk}+$). F_1's from such a cross, known to be *Fu/T*, were selected for matings to the C57BL/6 strain. Progeny tests of possible *Fu/T* animals were necessary since classification of these animals by tail type was not always certain. Seventy-eight F_1 animals were raised. Of the 21 animals tested only 5 proved to be both *Fu* and *T*.

The remainder carried either *Fu* or *T* only. In most animals proven *Fu*/*T* an extreme tail type was found; however, in one case, the tail was more typical of animals bearing *T* only.

Mice of the BC generation were saved and tested genetically by matings (1), (2) and (3). This was necessary because "false negatives" and "false positives" (to be described later) occur and tend to obscure results expected of particular genomes. At least 10 of the progeny of each BC mouse were inoculated, these mice being referred to as the testcross generation or TC. From the reaction of the TC mice in a particular progeny to the tumor inoculated, the genotype of the heterozygous BC parent could be determined. The number of TC young necessary to establish the parent's genotype beyond doubt varied from case to case, being dependent on both genetic and environmental conditions. As a rule 10 to 20 TC mice sufficed to prove a particular BC mouse a noncrossover. In a few cases TC young had to be saved and mated when testing of a BC mouse was incomplete or the results were open to question. In cases involving crossovers many more young were raised and inoculated. Two hundred ninety-five BC mice were mated, but, of these, 11 either died before sufficient data had been obtained or proved to be sterile, so that only 284 mice were actually tested.

It would have been desirable to have confined crosses to matings (2) and (3), but space and time did not permit this. Matings (2) and (3) are complementary tests for the possible separation of components *d* and *k*. In the case of a BC mouse in which separation had occurred, one but not the other of (2) and (3) would give tumor susceptible offspring. By the same BC mouse mating (1) would give young negative to the tumor. Thus, where (1) was not used, it was absolutely necessary to use both (2) and (3), otherwise the results might be misleading. Only one class was not tested for component separation; namely, noncrossover fused BC males.

For the classification of tail types, litters were inspected both at birth and at weaning. At weaning, profile drawings of the tails of all mice were made. For TC mice such classification was made "blind," i.e., without knowledge of the recorded tail type of the BC parent. This served to exclude any possible subjective bias in the classification.

TC mice were inoculated by trocar at 5 to 12 weeks of age and checked weekly thereafter, as described in the section on materials and methods.

Class	Alternative 1 $\frac{Fu}{+}\ ^{xa}\ \frac{H\text{-}2^f}{H\text{-}2^{dk}}\ ^{xb}\ \frac{+}{T}$				Alternative 2 $\frac{H\text{-}2^f}{H\text{-}2^{dk}}\ ^{xa}\ \frac{Fu}{+}\ ^{xb}\ \frac{+}{T}$				Alternative 3 $\frac{H\text{-}2^f}{H\text{-}2^{dk}}\ ^{xa}\ \frac{+}{T}\ ^{xb}\ \frac{Fu}{+}$			
	nc	*xa*	*xb*	*dc*	*nc*	*xa*	*xb*	*dc*	*nc*	*xa*	*xb*	*dc*
Fu	−			+	−	+			−	+		
T	+			−	+	−			+	−		
FuT		+	−				−	+			+	−
+		−	+				+	−			−	+

FIGURE 3.—Pattern of response to inoculation of strain A tumor 15091a under three alternative orders of the loci *Fu*, *T* and *H-2*. Noncrossover = nc; crossover at region a = xa; crossover at region b = xb; double crossover = dc. Resistant to 15091a = −; susceptible to 15091a = +.

The pattern of response expected of the BC generation for each of the three possible alternative orders of the loci *Fu*, *T* and *H-2* is diagrammed in figure 3, which summarizes the classes expected under each of the alternatives. There are key classes which differentiate one alternative from the other two so that the most probable order of the loci may be established.

RESULTS

Penetrance of tail genes (*See table 2*)

In both the crosses involving *T* or *Fu* the total mortality between birth and weaning of TC mice was greatest when matings of BC parents were made to the C57BL/6 strain. The total mortality was less in matings to the AKR strain and least to the BALB/c strain. Since the BC generation was half C57BL/6 in genetic background, differences in mortality are probably explained on the basis of an increased homozygosity of the young in the first mating and an increased heterozygosity in the latter two matings. In almost all cases the total mortality was higher when *T* was segregating than when *Fu* was segregating. When the female parent was of a pure strain, the mortality was greater than when the female parent was heterozygous.

In all crosses involving *T*, the ratios obtained at birth of *T* to + very closely approximated the expected 1:1 ratio. At weaning differential mortality was evident

TABLE 2

Penetrance of T *and* Fu *by age, sex and strain*

Strain used in cross	Sex of BC parent	No. at birth			No. at weaning			% *T*	% Mortality		
		T	+	Total	*T*	+	Total		*T*	+	Total
1a C57BL/6	♂	992	994	1986	699	773	1472	47.5	14.8	11.1	25.9
b "	♀	772	761	1533	642	678	1320	48.6	8.5	5.4	13.9
2a BALB/c	♂	86	67	153	74	54	128	57.8	7.8	8.5	16.3
b "	♀	113	99	212	105	91	196	53.6	3.8	3.8	7.6
3a AKR	♂	49	50	99	31	45	76	40.8	18.2	5.1	23.2
b "	♀	126	130	256	111	129	240	46.3	5.9	0.4	6.3

Strain used in cross	Sex of BC parent	No. at birth			No. at weaning			% *Fu*	Mortality % Total	% *Fu* matings compared	χ^{2*}	P
		Fu	+	Total	*Fu*	+	Total					
1a C57BL/6	♂	895	1344	2239	768	1042	1810	42.4	19.2	1a & b	158.2	<.0001
b "	♀	15	418	433	24	326	350	6.86	19.2	1b & 2b	91.6	<.0001
2a BALB/c	♂	80	142	222	73	131	204	35.8	8.1	2a & b	0.48	.50
b "	♀	327	831	1158	370	751	1121	33.0	3.2	2b & 3b	35.5	<.0001
3a AKR	♂	52	128	180	50	101	151	33.1	16.1	3a & b	8.78	<.01
b "	♀	202	1084	1286	269	956	1225	22.0	4.7	1b & 3b	40.0	<.0001

* Yates' correction applied.

in crosses to the AKR and C57BL/6 strains. With one parent from the AKR strain, *T* offspring are heavily selected against. A slight differential mortality is observed with the C57BL/6 strain. However, with one parent from the BALB/c strain, there appears to be no particular selection against *T*; animals carrying *T* or + are eliminated at equal rates.

It is not possible to measure the amount of differential mortality in crosses segregating for *Fu* because the expression of *Fu* at birth is often so slight that such animals may be classified as normal-tailed. Thus, the absolute number of recognized *Fu* is greater at weaning. There are both strain and sex differences in the penetrance of *Fu*. "Normal overlaps" are generally higher in crosses to certain strains. Where the female is the fused parent, there are very significant differences in the penetrance of *Fu* found in different strains. For the strains used the percent penetrance was: C57BL/6—6.86%; BALB/c—33%; and AKR—22%; where 50% is the expected value. There are significant differences in the penetrance of *Fu* between male and female in crosses to the C57BL/6 strain (42.4% *vs.* 6.86%) and in crosses to the AKR strain (33.1% *vs.* 22.0%) but not in crosses to the BALB/c strain (35.8% *vs.* 33.0%). The number of normal overlaps was so great in the mating of heterozygous females to C57BL/6 males that it had to be abandoned in favor of matings to BALB/c and AKR males.

Noncrossovers

Data obtained from noncrossovers are grouped by tail class, sex and mating in table 3. The results of tumor inoculation of the TC generation are totaled for each group. The first four subcolumns represent: (1) normal-tailed positive to A-strain tumor 15091a (++); (2) normal-tailed negatives (+−); (3) brachy-tailed (or fused-tailed) positives (*T*+); and (4) brachy-tailed (or fused-tailed) negatives (*T*−). Then follow two subcolumns representing total young positive (+) and total

TABLE 3

Data on noncrossovers

Classification of BC mice	Mating	Results of tumor inoculation of TC young					
		++	+−	*T*+	*T*−	+	−
62 *Fu* ♂♂	♀C57BL/6	1	968	0	715	1	1683
	♀BALB/c	0	49	0	27	0	76
	♀AKR	3	19	0	14	3	33
72 *Fu* ♀♀	♂C57BL/6	2	317	0	24	2	341
	♂BALB/c	3	697	0	361	3	1058
	♂AKR	10	906	2	265	12	1171
65 *T* ♂♂	♀C57BL/6	51	671	517	142	568	813
68 *T* ♀♀	♂C57BL/6	66	509	348	205	414	714
	♂BALB/c	4	41	46	7	50	48
	♂AKR	15	50	43	9	58	59

young negative (−). Individual progenies of BC animals within each tail class were tested by chi square for fit to the total values from all progenies of the class.

Fused noncrossovers are expected to produce offspring which are all negative to A-strain tumor 15091a no matter which alternative arrangement of the loci proves correct. The observed results show that this is the case with one qualification. "False positives" occur. These are animals genetically resistant to a tumor but which succumb to it due to a combination of residual heredity plus environmental factors of a largely unknown nature. In the present crosses there were relatively few "false positives" occurring in the fused-tail noncrossover class. The percentage of false positives varied according to the pure strain used in the matings. The frequency of false positives was highest in crosses to AKR (♂ and ♀ *Fu* 15/1219 = 1.23%), next in crosses to BALB/c (♂ and ♀ *Fu* 3/1137 = 0.26%) and lowest in crosses to C57BL/6 (♂ and ♀ *Fu* 3/2027 = 0.10%).

The brachy noncrossover group is an extremely interesting one because it affords a second measure of crossing over between *H-2* and *T*. Under any of the alternative hypothetical orders of the three loci under consideration, brachy noncrossovers should throw *T* positive young. All matings of a BC *T* mouse may be expressed as the following: $H\text{-}2^{dk}T/H\text{-}2^{b} \times H\text{-}2^{z}+/H\text{-}2^{z}+$. Thus, the noncrossover TC young expected would be normal-tailed negatives and brachy positives; crossovers would be normal-tailed positives and brachy negatives. That is exactly what is observed in the results of the inoculation of TC young by *T* noncrossover parents. Subcolumns (2) and (3) contain noncrossovers while (1) and (4) include crossovers (table 3).

In the crosses to C57BL/6 it soon became obvious that there were more negative animals in class (4) (*T*−) than expected; that is, the number of "false negatives" was high. Also, the number of negatives appeared to be higher in the first lots of mice tested than in the later ones. The data were therefore examined by month of inoculation, with the results depicted graphically in figure 4. The abscissa represents the percent positive to 15091a, while the ordinate represents the months of inoculation from February 1952 to July 1953. The total data are graphed as well as data from *T* TC young from heterozygous males and from heterozygous females. It may be observed that data from total animals fall below the expected 50 percent positive line and that data from *T* TC mice generally fall between 50 and 100 percent positive. It may also be noted that there is a marked decrease in positive animals during the summer months of 1952.

The tumor repeatedly became infected during these months; therefore, a switch was made on October 17, 1952 from the trocar technique of transplantation to the cytosieve technique in carrying the tumor in the donor line. Using the latter technique, the tumor was always free from infection. A consequent upward swing in percent positives may be observed. Similar reductions in the growth potential of transplanted tumors due to infection have been reported elsewhere (see, e.g., SCHREK 1936).

For comparing the effects of the two techniques of transplantation, the date, December 1, 1952, was chosen for dividing the data because of an initial retardation in tumor growth rate when the medium employed for tumor suspension was changed in these experiments. The number of positives produced by all BC *T* noncrossovers

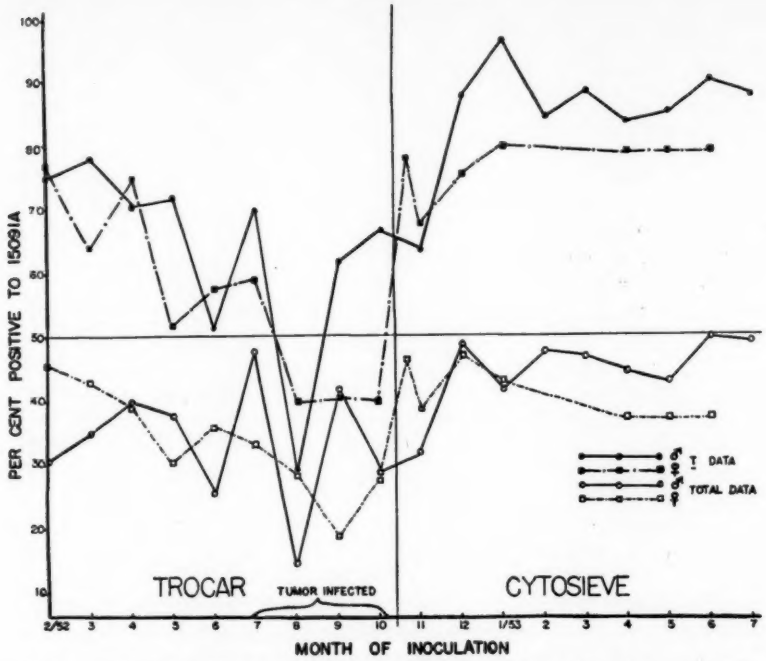


FIGURE 4.—Percent positive to strain A tumor 15091a by month of inoculation among TC offspring of *T* noncrossovers × C57BL/6. Both the percent positive of the total animals inoculated and the percent positive of *T* animals are represented for each sex. 15091a was carried in the donor line by trocar before 10/17/52. After this date the cytosieve was used.

before versus after December 1, 1952 was analyzed statistically, and a very significant difference was found between the two populations (P = <.0001).

Previous to December 1, 1952 it was found that the age at inoculation had a slight influence on the number of positives. The number of positives was less among animals inoculated at 2 or more months of age than among those inoculated earlier (P = <.01). After December 1, 1952, most of the mice were inoculated at an earlier age. A comparison of the number of positives among mice inoculated at 1½ months or older and among those inoculated earlier showed that the age effect was lost (P = .50).

When calculating crossover percents, it was necessary to take into account "false negatives." A method was devised for determining the proportion of "false negatives" among *H-2*^{dk} mice in the various divisions of *T* noncrossover data and then calculating the frequency of crossing over or "*c*". The method used was the following:

(a) Let q = actual proportion of brachy
x = proportion of false negatives
c = frequency of recombination

(b) For each of the four classes (1) through (4) the absolute numbers were listed and the percentage contribution calculated. Algebraic expressions for the theoretical frequencies are shown for each class.

Classes	Theoretical frequency
(1) $++$	$(1-q)c-(1-q)cx$
(2) $+-$	$(1-q)(1-c)+(1-q)cx$
(3) $T+$	$q(1-c)-q(1-c)x$
(4) $T-$	$qc+q(1-c)x$

$$\text{(c)}\quad x=\frac{(2)+(4)-(1)-(3)-(1-2q)(1-2c)}{1-(1-2q)(1-2c)}$$

$$\text{(d)}\quad c=\frac{(1)+(4)-qx}{(1-x)}$$

Table 4, part 1 summarizes the results of examining various crosses to the C57BL/6 strain for factors instrumental in altering the frequency of false negatives. Data divided at December 1, 1952 for heterozygous males and females show in both Cases 1 and 2 a higher frequency of false negatives before than after this date. In Case 1 there were 30.6 percent negatives before December 1, 1952 and 4 percent negatives after this date. In Case 2 the corresponding percentages are 28.9 and 15.3.

An influence of age at inoculation of TC young on the percentage of false negatives is demonstrated in Cases 3 to 6. In general the frequency of false negatives was higher in the older age groups. The data are listed separately for males and for females. Cases 3 and 4 group data obtained before December 1, 1952; while Cases 5 and 6 group data obtained after this date.

For any line of data in Cases 1 to 7 the values of q, x and c may be substituted in the theoretical frequencies (1) to (4). These give the exact observed frequencies, since there are no degrees of freedom, and are of interest in showing the extent of agreement from wholly independent determinations.

As indicated at the foot of table 4, a few T animals negative to the tumor or not inoculated were proved by matings to C57BL/6 (or BALB/c and AKR) to be genetically positive to the tumor. These were either not counted or not counted positive in Cases 1 to 6, already discussed. With the attempt to establish a sex difference in crossing over these animals were counted as positives in the remaining cases to be discussed. In this respect Case 7 is a revision of Cases 1 and 2. There is actually very little change in the calculated values of x and c.

Part 2 of table 4 is an attempt to demonstrate a sex influence on crossing over. All cases start with the observed frequencies found in Case 7. It was assumed first (Case 8) that there is a constant value of c in each sex, whether before or after December 1, 1952, but that the values of x may be different in the sexes, both "before" and "after". The final estimates were obtained by iteration between equations (c) and (d) using the weighted averages of the c's "before" and "after" at each step until stability was reached. These estimates yield a total chi square of 3.448 with 2 degrees of freedom and a probability between .10 and .20. This hypothesis thus gives reasonably satisfactory results.

TABLE 4

Factors influencing the frequency of false negatives and crossovers produced by BC T noncrossovers mated to C57BL/6

PART 1

Influence of Age at Inoculation and Method of Inoculation

Case No.	Sex	Age at inoc.	Trocar vs. cytosieve ± 12/1/52	Response to tumor 15091a					Frequency of:	
				++	+−	T+	T−	N	x	c
1.	♂		Before	17	286	165	95	545	.3057	.0859
			After	34	403	351	47	835	.0402	.0811
2.	♀		Before	61	448	286	197*[7]	992	.2880	.1684
			After	5	61	54	16*[1]	136	.1527	.0895
3.	♂	2 mos.+	Before	11	176	114	77	378	.3443	.0897
		−2 mos.		6	92	51	18	167	.1997	.0765
4.	♀	2 mos.+	Before	51	388	221	177*[6]	837	.3287	.1730
		−2 mos.		10	60	65	20*[1]	155	.0924	.1574
5.	♂	1½ mos.+	After	3	72	56	7	138	.0711	.0430
		−1½ mos.		31	331	295	40	697	.0338	.0887
6.	♀	1½ mos.+	After	1	31	31	11*[1]	74	.2307	.0406
		−1½ mos.		4	30	23	5	62	.0609	.1252
7.	♂		Before	17	268	166†[1]	95	546	.3043	.0858
			After	34	403	351	47	835	.0403	.0811
	♀		Before	61	448	293*[7]	190	992	.2735	.1650
			After	5	61	55*[1]	15	136	.1385	.0879

PART 2

Influence of Sex on Crossing Over

Case No.	Sex	Trocar vs. cytosieve 12/1/52	N	Frequency of:		x^2		n	P
				z	c				
8.	♂	Before	546	.3041	.0829	.025	.058	1	.80–.90
		After	835	.0405	.0829	.033			
	♀	Before	992	.2732	.1560	.305	3.390	1	.05–.10
		After	136	.1352	.1560	3.085			
							3.448	2	.10–.20
9.	♂	Before	546	.2842	.0839	.452	4.385	4	.30–.50
		After	835	.0538	.0839	.798			
	♀	Before	992	.2842	.1561	.352			
		After	136	.0538	.1561	2.783			
10.	♂	Before	546	.3057 ± .0321	.0825 ± .0103	.031	.044	1	.80–.90
		After	835	.0390 ± .0198	.0825 ± .0103	.013			
	♀	Before	992	.2782 ± .0261	.1541 ± .0157	.414	2.790	1	.05–.10
		After	136	.0904 ± .0595	.1541 ± .0157	2.376			
							2.834	2	.20–.30

* Negative when inoc.; tested genetically as +.

† Not inoc.; tested genetically as +; counted here only.

TABLE 4—*Continued*

PART 3

Method for estimating values of c, x_a, and x_b by the Method of Maximum Likelihood. The derivations of formulae for the first and second derivatives of c, x_a and x_b with respect to L are shown, where $L = \log P$

A Class	B p = theoretical proportions (separating terms in c)	C $\frac{\partial p}{\partial c}$	D $\partial \frac{\log p}{\partial c} = \frac{1}{p}\frac{\partial p}{\partial c}$	E $\frac{\partial^2 p}{\partial c^2}$	F $\frac{\partial^2 p}{\partial x \partial c}$	G = C × D $\frac{1}{p}\left(\frac{\partial p}{\partial c}\right)^2$
++	$p_1 = (1-q)(1-x)c$	$(1-q)(1-x)$	$\frac{1}{c}$	0	$-(1-q)$	$\frac{(1-q)(1-x)}{c}$
+−	$p_2 = (1-q) - (1-q)(1-x)c$	$-(1-q)(1-x)$	$-\frac{1-x}{1-(1-x)c}$	0	$(1-q)$	$\frac{(1-q)(1-x)^2}{1-(1-x)c}$
T+	$p_3 = q(1-x) - q(1-x)c$	$-q(1-x)$	$-\frac{1}{1-c}$	0	q	$\frac{q(1-x)}{1-c}$
T−	$p_4 = qx + q(1-x)c$	$q(1-x)$	$\frac{1-x}{x+(1-x)c}$	0	$-q$	$\frac{q(1-x)^2}{x+(1-x)c}$

A′ Class	B′ p = theoretical proportions (separating terms in x)	C′ $\frac{\partial p}{\partial x}$	D′ $\partial \frac{\log p}{x} = \frac{1}{p}\frac{\partial p}{\partial x}$	E′ $\frac{\partial^2 p}{\partial x^2}$	F′ = F $\frac{\partial^2 p}{\partial c \partial x}$	G′ = C′ × D′ $\frac{1}{p}\left(\frac{\partial p}{\partial x}\right)^2$	H′ = D × C′ = D′ × C $\frac{1}{p}\left(\frac{\partial p}{\partial c}\right)\left(\frac{\partial p}{\partial x}\right)$
++	$p_1 = (1-q)c - (1-q)cx$	$-(1-q)c$	$-\frac{1}{1-x}$	0	$-(1-q)$	$\frac{(1-q)c}{1-x}$	$-(1-q)$
+−	$p_2 = (1-q)(1-c) + (1-q)cx$	$(1-q)c$	$\frac{c}{(1-c)+cx}$	0	$(1-q)$	$\frac{(1-q)c^2}{(1-c)+cx}$	$-\frac{(1-q)(1-x)c}{1-(1-x)c}$
T+	$p_3 = q(1-c) - q(1-c)x$	$-q(1-c)$	$-\frac{1}{1-x}$	0	q	$\frac{q(1-c)}{1-x}$	q
T−	$p_4 = qc + q(1-c)x$	$q(1-c)$	$\frac{1-c}{c+(1-c)x}$	0	$-q$	$\frac{q(1-c)^2}{c+(1-c)x}$	$\frac{q(1-x)(1-c)}{x+(1-x)c}$

$$\sum p_i = 1$$

(1) $$L = \sum_{i=1}^{4} \{n_{ia} \log p_{ia}\} + \sum_{i=1}^{4} \{n_{ib} \log p_{ib}\}, \text{ where } \begin{cases} n_{ia} = \text{observed no. in class } ia \\ n_{ib} = \text{observed no. in class } ib \end{cases}$$

(2) $$\frac{\partial L}{\partial c} = \sum_{i=1}^{4} \left\{ n_{ia} \frac{1}{p_{ia}} \frac{\partial p_{ia}}{\partial c} \right\} + \sum_{i=1}^{4} \left\{ n_{ib} \frac{1}{p_{ib}} \frac{\partial p_{ib}}{\partial c} \right\} = 0$$

(3) $$\frac{\partial^2 L}{\partial c^2} = \sum_{i=1}^{4} \left\{ \bar{n}_{ia} \frac{1}{p_{ia}^2} \left[P_{ia} \frac{\partial^2 p_{ia}}{\partial c^2} - \left(\frac{\partial p_{ia}}{\partial c} \right)^2 \right] \right\} + \sum_{i=1}^{4} \left\{ \bar{n}_{ib} \frac{1}{p_{ib}^2} \left[p_{ib} \frac{\partial^2 p_{ib}}{\partial c^2} - \left(\frac{\partial p_{ib}}{\partial c} \right)^2 \right] \right\}$$

(4) $$= - \sum_{i=1}^{4} \left\{ \bar{n}_{ia} \left[\frac{1}{p_{ia}} \left(\frac{\partial p_{ia}}{\partial c} \right) \right]^2 \right\} - \sum_{i=1}^{4} \left\{ \bar{n}_{ib} \left[\frac{1}{p_{ib}} \left(\frac{\partial p_{ib}}{\partial c} \right) \right]^2 \right\} \quad \text{since } \frac{\partial^2 p_{ia}}{\partial c^2} = \frac{\partial^2 p_{ib}}{\partial c^2} = 0$$

(5) $$= -N_a \sum_{i=1}^{4} \left[\frac{1}{p_{ia}} \left(\frac{\partial p_{ia}}{\partial c} \right)^2 \right] - N_b \sum_{i=1}^{4} \left[\frac{1}{p_{ib}} \left(\frac{\partial p_{ib}}{\partial c} \right)^2 \right] \quad \begin{matrix} \text{since } \bar{n}_{ia} = N_a p_{ia}, \bar{n}_{ib} = N_b p_{ib} \\ \text{where } N_a = \sum_{i=1}^{4} n_{ia}, N_b = \sum_{i=1}^{4} n_{ib} \end{matrix}$$

(6a) $$\frac{\partial L}{\partial x_a} = \sum_{i=1}^{4} \left\{ n_{ia} \frac{1}{p_{ia}} \frac{\partial p_{ia}}{\partial x_a} \right\} = 0$$

(6b) $$\frac{\partial L}{\partial x_b} = \sum_{i=1}^{4} \left\{ n_{ib} \frac{1}{p_{ib}} \frac{\partial p_{ib}}{\partial x_b} \right\} = 0$$

(7a) $$\frac{\partial^2 L}{\partial x_a^2} = -N_a \sum_{i=1}^{4} \left[\frac{1}{p_{ia}} \left(\frac{\partial p_{ia}}{\partial x_a} \right)^2 \right]$$

(7b) $$\frac{\partial^2 L}{\partial x_b^2} = -N_b \sum_{i=1}^{4} \left[\frac{1}{p_{ib}} \left(\frac{\partial p_{ib}}{\partial x_b} \right)^2 \right]$$

(8a) $$\frac{\partial^2 L}{\partial x_a \partial c} = \sum_{i=1}^{4} \left\{ \bar{n}_{ia} \frac{1}{p_{ia}^2} \left[p_{ia} \frac{\partial^2 p_{ia}}{\partial x_a \partial c} - \left(\frac{\partial^2 p_{ia}}{\partial c} \right) \left(\frac{\partial p_{ia}}{\partial x_a} \right) \right] \right\}$$

(8b) $$\frac{\partial^2 L}{\partial x_b \partial c} = \sum_{i=1}^{4} \left\{ \bar{n}_{ib} \frac{1}{p_{ib}^2} \left[p_{ib} \frac{\partial^2 p_{ib}}{\partial x_b \partial c} - \left(\frac{\partial p_{ib}}{\partial c} \right) \left(\frac{\partial p_{ib}}{\partial x_b} \right) \right] \right\}$$

(9a) $$= N_a \sum_{i=1}^{4} \left[\frac{\partial^2 p_{ia}}{\partial x_a \partial c} - \frac{1}{p_{ia}} \left(\frac{\partial p_{ia}}{\partial c} \right) \left(\frac{\partial p_{ia}}{\partial x_a} \right) \right]$$

(9b) $$= N_b \sum_{i=1}^{4} \left[\frac{\partial^2 p_{ib}}{\partial x_b \partial c} - \frac{1}{p_{ib}} \left(\frac{\partial p_{ib}}{\partial c} \right) \left(\frac{\partial p_{ib}}{\partial x_b} \right) \right]$$

(10) $$\frac{\partial^2 L}{\partial x_a \partial x_b} = 0$$

TABLE 4—*Continued*

PART 4

Formulae for the standard errors of c, x_a and x_b

$$\sigma c^2 = -\frac{Dcc}{D}, \qquad \sigma x_a^2 = -\frac{D_{x_a x_a}}{D}, \qquad \sigma x_b^2 = -\frac{D_{x_b x_b}}{D}$$

where

$$D = \begin{vmatrix} \frac{\partial^2 L}{\partial c^2} & \frac{\partial^2 L}{\partial x_b \partial c} & \frac{\partial^2 L}{\partial x_a \partial c} \\ \frac{\partial^2 L}{\partial x_b \partial c} & \frac{\partial^2 L}{\partial x_b^2} & \frac{\partial^2 L}{\partial x_b \partial x_a} \\ \frac{\partial^2 L}{\partial x_a \partial c} & \frac{\partial^2 L}{\partial x_b \partial x_a} & \frac{\partial^2 L}{\partial x_a^2} \end{vmatrix}$$

and D_{cc}, $D_{x_a x_a}$, $D_{x_b x_b}$ are the minors indicated by the subscripts.

PART 5

Difference/S.E. Difference of the frequency of crossovers and false negatives

$$\frac{c_{♀} - c_{♂}}{\sigma c_{♀} - \sigma c_{♂}} = \frac{.0716}{.0188} = 3.809 \qquad \frac{x_{b♂} - x_{b♀}}{\sigma x_{b♂} - \sigma x_{b♀}} = \frac{.0275}{.0414} = .664 \qquad \frac{x_{a♀} - x_{a♂}}{\sigma x_{a♀} - \sigma x_{a♂}} = \frac{.0514}{.0626} = .821$$

$$\frac{x_{b♂} - x_{a♂}}{\sigma x_{b♂} - \sigma x_{a♂}} = \frac{.2667}{.0354} = 7.534 \qquad \frac{x_{b♀} - x_{a♀}}{\sigma x_{b♀} - \sigma x_{a♀}} = \frac{.1878}{.0649} = 2.894$$

Estimates were also obtained (Case 9) on the hypothesis that x is the same in males and females before December 1, 1952 and similarly for x after this date, as well as that there are single values of c within each sex. Weighted averages were used for x_a, x_b, c_{σ^*} and c_{φ} in the iteration process. Chi square came out slightly larger (4.385) but as there are now 4 degrees of freedom, the probability is raised to the .30 to .50 category for the set as a whole. It is evident that these hypotheses account for the data very satisfactorily. They do not, however, give standard errors for the c's and x's. These must be considerably larger than if there were no false negatives ($\sigma_c^2 = c(1 - c)/N$), or no crossing over ($\sigma_{x_a}^2 = x_a(1 - x_a)/Nq_a$). The method of averaging used does not, moreover, give the best estimates, since no cognizance is taken of the effects of x and c on reliability.

The estimates with single c's for each sex were accordingly calculated by the method of maximum likelihood, iterating between the equations $\partial L/\partial c = 0$ and $\partial L/\partial x_a = 0$ and $\partial L/\partial x_b = 0$ (table 4, part 3). Standard errors were obtained by the method described by FISHER (1921). The formulae for the standard errors may be found in table 4, part 4. The results obtained by these methods are given in Case 10. Chi square is reduced to 2.834 which with 2 degrees of freedom gives a probability of .20 to .30 in comparison with .10 to .20 in Case 8. There is clearly a significant difference between males and females in recombination (males, .0825 ± .0103; females, .1541 ± .0157). Neither x_a nor x_b differs significantly between males and females but they differ significantly from each other in both sexes (table 4, part 5).

Crossovers

Data on 17 crossovers are listed in table 5. Positive and negative young, irrespective of tail type, are grouped according to mating for each crossover. Three classes of crossovers were definitely obtained and possibly a fourth, which were: fused positive (*Fu*+), brachy negative (*T*−), normal-tailed positive (++), and fused-brachy negative (*FuT*−). Chi square tests show that data from crossovers are clearly separated from data from the corresponding noncrossover groups.

The 3 males in the *Fu*+ group gave positive *Fu* young in all crosses. Since noncrossover *Fu* males mated to the C57BL/6 strain produced only 1 positive out of 1684 mice, the occurrence of positives in approximately half the mice in these crosses indicates crossing over between the loci *H-2* and *Fu*.

There are two cases that require special consideration because of an apparent separation of the *d* and *k* components. ♀ 539 (*Fu*) gave 6 positive and 5 negative young from a cross with BALB/c ($H\text{-}2^d/H\text{-}2^d$), as expected for a crossover ($H\text{-}2^{dk}Fu/H\text{-}2^b+$), but gave no positives, 9 negatives from a cross with AKR ($H\text{-}2^k/H\text{-}2^k$), as if she were a noncrossover ($H\text{-}2^fFu/H\text{-}2^b+$). These results could be taken into account by supposing (1) that she is a ($H\text{-}2^{dk}Fu$) crossover but that the number of young from the cross with AKR was so small that no positives happened to appear; or (2) that a separation of the *d* and *k* components occurred in a crossover that implies the order *K D Fu T* of the loci; or (3) that a mutation from $H\text{-}2^f$ to $H\text{-}2^k$ occurred. The first hypothesis seems the simplest and is adopted.

♂ 278 (*T*) gave 33 positives, 28 negatives from a cross with BALB/c, as expected for a noncrossover ($H\text{-}2^{dk}T/H\text{-}2^b+$), but gave no positives, 39 negatives from a cross

TABLE 5

Data on 17 crossovers

Class and no.	Results of tumor inoculation					
	C57BL/6		BALB/c		AKR	
	+	−	+	−	+	−
♂ *Fu*:						
290	11	11	21	25	16	23
346	20	21	16	25	23	24
529	12	22	20	19	15	14
♀ *Fu*:						
539			6	5	0	9
♂ *T*:						
278	0	19	33	28	0	39
356	0	37	1	51	0	31
♀ *T*:						
220	1	48	0	16	0	15
331	0	27	0	22	0	21
364	0	22	0	25	2	27
491	0	37	0	25	1	30
188	0	20	0	10	1	15
♂ *FuT*:						
521	0	30	0	47	6*	41
♂ +:						
336	29	46	33	25	14	11
465	18	16	13	7	12	13
♀ +:						
274	12	6	16	18	22	23
379	8	8	14	19	23	15
485	3	1	19	25	19	14

* Genetically resistant on basis of long survival.

with AKR, as if he were a crossover ($H\text{-}2^{f}T/H\text{-}2^{b}+$) or at least lacked the *d* component. The cross to C57BL/6 ($H\text{-}2^{b}/H\text{-}2^{b}$) gives further support to this hypothesis (0 positives, 19 negatives). In this case, chance absence of positives from these two crosses may be ruled out. The results can be accounted for most simply either by a separation of the *d* and *k* components in a crossover, that, in this case, implies the order *D K Fu T*, or by a mutation from $H\text{-}2^{dk}$ to $H\text{-}2^{k}$ in a noncrossover.

There were 6 other *T* crossovers, 1 male and 5 females. Almost all *T* offspring were negative to the tumor, such positives as did occur being of the frequency of "false positives". Whether some of these crossovers also carried *Fu* could not be determined. The tail types were typically *T*, but *Fu* may also have been present.

Five normal-tailed crossovers were found, half of whose young were positive to 15091a. The genotype of this class was thus $H\text{-}2^{dk}+/H\text{-}2^{b}+$. In this class there were 2 males and 3 females. Large numbers of offspring were raised in order to exclude the possibility that any of these normal-tailed animals were actually normal overlaps (genetically *Fu*). A chi square test of the total number of normal-tailed young pro-

duced by suspected normal-tailed crossovers as compared with the total array of progeny produced by *Fu* mice, of each sex and mating type, revealed a significant difference in every case. Data on the results of tumor inoculation from all matings were pooled for each normal-tailed mouse. A good fit to a 1:1 ratio was found when t-tests were run.

A very real drawback in testing the genotypic array of the BC generation was the lack of a decisive test for the *FuT* class. There should have been some animals of this genotype unless they suffered a heavy mortality. There were 5 normal-tailed positives in 284 BC mice, so mice containing the complementary chromosome, *H-2^{t}FuT*, would be expected. These animals should produce all negative young. Of course, the possibility remains that this class could have been confused with two other classes, *Fu* noncrossovers or, more probably, *T* crossovers.

Male 521, suspected of being *FuT*, was subjected to rigorous testing. Despite the production of 57 offspring with affected tails, unequivocal classification as *FuT* or *T* was impossible. But, whatever the genotype of ♂521, the inoculation results indicate that he was negative to the tumor because all his offspring were negative, with the exception of certain offspring of AKR parentage. The expected number of "false positives" here is .012. More were found probably for the reason that these mice were inoculated at weaning (4 weeks). A true genetically resistant state is suggested because the tail-affected positive and 3 of the 5 normal-tailed positives survived an average of 12 or more weeks before dying, a time interval of some 7 weeks longer than the mode for animals which are genetically positive.

Finally, mention should be made of the distribution of crossovers among progenies of the *Fu/T* animals used in producing the BC generation. Little individual variation was evident in the male data, for the frequency of crossovers found in any one progeny approximated that found in the total data. Among 9 offspring produced by the one *Fu/T* female was one crossover.

Order of the loci established

The most probable order of the three loci is *H-2 Fu T*. Such a conclusion becomes apparent when observed and expected classes under each possible alternative arrangement of the three loci are compared (See figure 3 for expected classes). The key classes making the second alternative seem most likely are the occurrence of *Fu*+ and ++ mice. Finding *Fu*+ mice excludes alternative 1 because these mice would then have to be considered double crossovers. By the same reasoning, finding animals which are genetically ++ excludes alternative 3.

Table 6 listing the observed and calculated values for each of the three alternatives demonstrates the close agreement between these values for alternative 2. There, the genotype of ♂521 is considered *Fu T*. Very little difference is found in the chi square values if ♂521 is considered as *T* only. To include the greatest number of expected double crossovers, no interference of the loci was assumed. This is the most unfavorable assumption. Thus, the calculated double crossovers are the product of the total crossovers at region a times the total crossovers at region b. The results of chi square tests show a probability of $<.0001$ for alternatives 1 or 3 and a probability of .70 for alternative 2. It may be concluded that the order of the loci is *H-2 Fu T*.

TABLE 6

Observed and calculated values establishing the order of the three loci as H-2 Fu T

Classes	Alternative 1 Fu^{+a} $H\text{-}2^{+b}T$		Alternative 2 $H\text{-}2^{+a}Fu^{+b}T$		Alternative 3 $H\text{-}2^{+a}$ T^{+b} Fu	
	Observed	Calculated	Observed	Calculated	Observed	Calculated
Noncrossovers	267	256.66	267	267.25	267	261.36
Singles at (a)	0	10.33	11	10.76	11	16.65
Singles at (b)	6	16.35	6	5.76	0	5.63
Doubles	11	0.66	0	0.23	6	0.36
Total	284	284.00	284	284.00	284	284.00
	Fu—H-2 = 0.0387 (a) *H-2——T* = 0.0599 (b) *Fu——T* = 0.0986 (a) × (b) = 0.002318 (a) − 0.002318 = 0.036382 (b) − 0.002318 = 0.057582		*H-2—Fu* = 0.0387 (a) *Fu——T* = 0.0211 (b) *H-2——T* = 0.0599 (a) × (b) = 0.000817 (a) − 0.000817 = 0.037883 (b) − 0.000817 = 0.020283		*H-2——T* = 0.0599 (a) *T——Fu* = 0.0211 (b) *H-2—Fu* = 0.0810 (a) × (b) = 0.001264 (a) − 0.001264 = 0.058636 (b) − 0.001264 = 0.019836	
$2 \times 2\chi^{2*}$ =	152.8	P < .0001	0.2443	P = .70	71.3	P < .0001

* Yates' correction applied.

DISCUSSION

Three topics will be discussed: (1) Sex differences in crossing over; (2) Observed crossover values and the order of the loci; and (3) The nature of the *H-2* locus.

(1) The usual, but not invariable rule in the mouse is that crossing over is higher in the heterozygous female than in the heterozygous male (See review in GRÜNEBERG 1952). Chromosome IX is one of the chromosomes that conforms to this rule. DUNN and CASPARI (1945) found a probably significant difference between males and females for the interval *Ki-T*. In crosses where the heterozygous parent was *Ki*+/+*T* there was 14/1128 normal-tailed crossovers produced by male heterozygotes and 16/542 by female heterozygotes. The differences between the percentages 1.24 ± 0.33 and 2.95 ± 0.73 was on the borderline of being significant (P = <.04). Data from early experiments of DUNN and CASPARI (1945), which were designed to test recombination between the loci *Fu* and *Ki*, can be analyzed by sex. It is found that the 5 crossovers observed were all progeny of female heterozygotes.

Recently, DUNN and GLUECKSOHN-WAELSCH (1954) found no recombinants in a cross between *Fu* and *Ki* among 971 mice. Only 79 of these mice were heterozygous females. It may be that a sex difference in crossing over would explain the various conflicting results with these two genes. It is suggested that recombination is virtually 0 in males for this chromosomal interval but that it may take place in heterozygous females.

In crosses demonstrating *H-2^f* as a tenth allele at the *H-2* locus (ALLEN 1955), the observed values for the *H-2-Fu* interval were 6.85 percent for female heterozygotes and 4.1 percent for the males. Though the difference is not significant, the higher value again occurs in the female. In the experiments reported here a very significant sex difference in recombination was found in the interval *H-2-T*. Approximately twice as many crossovers were produced by female BC mice (15.4 percent) as by males (8.25 percent).

(2) Of the various mapped intervals of the ninth chromosome (See table 1), the most accurately determined crossover percentage is that for *H-2* and *T*. There is fair agreement between the 6.0 percent found in the BC generation (table 6) and the 8.25 percent in the TC generation produced by male *T* noncrossovers (table 4, part 2). For *H-2* and *Fu* the map distance remains somewhat uncertain, but these loci are no doubt fairly close together. If *Fu* and *Ki* are alleles, we then have two estimates for the distance between *H-2* and *Fu*. For allele *Fu* the frequency of crossing over indicated in heterozygous males is 3.9 percent; whereas, for allele *Ki* the distance appeared shorter in two point crosses (1.02 percent). This may indicate that some of the *T* crossovers in the above three point cross were actually *FuT*, and, if so, would suggest a shorter interval between *H-2* and *Fu* and a longer interval between *Fu* and *T*.

The order of the loci established was *H-2 Fu T*. If information from three point crosses is pooled (from the above and from a cross performed by DUNN and CASPARI 1945), the only possible order for all four loci would be *H-2 Fu Ki T*. DUNN and CASPARI crossed *Ki T/Fu* × normal-tailed mice and obtained 167 progeny with abnormal tails and 18 with normal tails. Of 9 of these normal-tailed mice, 6 proved to be genetically +++. Assuming that these tests were adequate, *Ki* and *Fu* cannot be

alleles, and the gene order *Ki Fu T* is practically eliminated as a possibility, since all normal-tailed mice would necessarily have been produced by double crossing over. They favored either the order (1) *Ki T Fu* or (2) *Fu Ki T*. The first of these is eliminated by the above crosses with *H-2*.

(3) There was only one good case (♂278) suggesting a separation of the *d* and *k* components; however, mutation from *H-2*^{dk} to *H-2*^{k} may possibly have occurred. Mutations are not uncommon at the *H-2* locus (BORGES and KVEDAR 1952; BORGES, KVEDAR and FOERSTER 1954; FOERSTER and BORGES 1954). It is indeed unfortunate that supporting serological tests were not performed on this mouse as well as on the exceptional fused animal (♀ 539). Therefore, more conclusive evidence is needed for proof of the separation of the components. The detection of a genetic marker to the left of *H-2* would considerably facilitate such proof.

Thus, whether the *H-2* locus is composed of inseparable components, or of two or more pseudoalleles cannot be decided at present. Pseudoalleles are genes which are very closely linked, though separable, with similar phenotypic effect, and which characteristically exhibit certain patterns of physiological interaction (LEWIS 1951). These genes are usually associated with chromosomal "repeats" or sectional changes of the chromosome.

If such is the case with *H-2*, it will be particularly difficult to determine the rate of crossing over within the complex, since large numbers of mice would be required. Moreover, if *H-2* has a high mutation rate, it will be even more difficult to decide between a true crossover and a mutation having occurred for any particular mouse in question.

SUMMARY

1. Genetically tested crossovers were found in the interval *H-2-Ki* (or *Fu*^{Ki}). Recombination occurred in 2/196 mice (1.02 percent), all offspring of heterozygous males.

2. Crosses were set up to test the order of the loci *Fu*, *T* and *H-2*. By genetic tests, where offspring were inoculated with strain A tumor 15091a, 17/284 BC mice were proven crossovers. The pattern of response of the crossovers indicated that the order of the loci is *H-2 Fu T*. If *Ki* is a separate locus from *Fu*, then the only possible order for all four loci is *H-2 Fu Ki T*.

3. Statistically significant differences were found between TC groups produced by the *T* noncrossover class mated to the C57BL/6 strain due to the following factors:

a. Inoculation technique. False negatives were lower after December 1, 1952 than before. The cytosieve was used for carrying 15091a in the donor line in the latter period, the trocar in the earlier period.

b. Age of animals inoculated. False negatives were slightly higher in older animals than in younger animals when the age division was ±2 months.

c. Sex difference in recombination in the interval *H-2-T*. After differences in false negatives were adjusted, recombination was found to be higher among offspring produced by heterozygous females than by heterozygous males. For males the crossover value was 8.25 percent, $N = 1381$; for females the value was 15.4 percent, $N = 1128$.

4. It has not been possible to determine whether the components at the *H-2* locus are separable. One crossover was found in which it appeared that the *d* component had been lost, the *k* component retained. However, the possibility of mutation from $H\text{-}2^{dk}$ to $H\text{-}2^{k}$ having occurred in this one case could not be ruled out.

ADDENDUM

GORER and co-workers (personal communication) have recently established two crossovers within the *H-2* locus by serological tests. Within this region there are possibly five loci, *DEFKC*, but their order is not yet certain. The first cross ($DE^{d}/D^{b}E$) resulted in a $D^{b}E^{d}$ crossover; this mouse was homozygous for *F* but has not been typed for *C*. The second cross ($DKC/D^{b}kc$) gave a $D^{b}KC$ crossover, the parents being homozygous for *E* and *F*. In the future, a revision in nomenclature for the "alleles" contained within the *H-2* region will undoubtedly be necessitated.

ACKNOWLEDGMENTS

I wish to extend my sincere gratitude to DR. GEORGE D. SNELL and to DR. SEWALL WRIGHT for their inspiration, guidance and criticism of the work reported. Credit is due DR. SEWALL WRIGHT for the mathematical method and derivation of formulae found in table 4.

LITERATURE CITED

ALLEN, SALLY LYMAN, 1955 *H-2f*, a tenth allele at the histocompatibility-2 locus in the mouse as determined by tumor transplantation. (Cancer Research **15:** 315–319).

BITTNER, J. J., 1935 A review of genetic studies on the transplantation of tumors. J. Genet. **31:** 471–487.

BORGES, P. R. F., and B. J. KVEDAR, 1952 A mutation producing resistance to several transplantable neoplasms in the C57 Black strain of mice. Cancer Research **12:** 19–24.

BORGES, P. R. F., B. J. KVEDAR, and G. E. FOERSTER, 1954 The development of an isogenic resistant subline of mice (C57BL/6-$H\text{-}2^{d}$) resistant to transplantable neoplasms indigenous to the C57BL strain. J. Nat. Cancer Inst. **15:** 341–346.

CLOUDMAN, A. M., 1932 A comparative study of the transplantability of eight mammary gland tumors arising in inbred mice. Am. J. Cancer **16:** 568–630.

DUNN, L. C., and E. CASPARI, 1945 A case of neighboring loci with similar effects. Genetics **30:** 543–568.

DUNN, L. C., and S. GLUECKSOHN-WAELSCH, 1953 The failure of a t-allele (t^{8}) to suppress crossing over in the mouse. Genetics **38:** 512–517.

1954 A genetical study of the mutation 'fused' in the house mouse, with evidence concerning its allelism with a similar mutation 'kink'. J. Genet. **52:** 383–391.

FISHER, R. A., 1921 On the mathematical foundations of theoretical statistics. Trans. Roy. Soc. London, A, **222:** 309–368.

FOERSTER, G. E., and P. R. F. BORGES, 1954 A subline of mice (DBA/1B), derived from the DBA/1 strain, resistant to transplantable tumors indigenous to the DBA/1 strain of mice. Transplantation Bull. **1:** 200–201.

GORER, P. A., 1936 The detection of antigenic differences in mouse erythrocytes by employment of immune sera. Brit. J. Exp. Pathol. **17:** 42–50.

1937 The genetic and antigenic basis of tumor transplantation. J. Pathol. Bacteriol. **44:** 691–697.

GORER, P. A., SALLY LYMAN, and G. D. SNELL, 1948 Studies on the genetic and antigenic basis of tumour transplantation: Linkage between a histocompatibility gene and 'fused' in mice. Proc. Roy. Soc. London, B, **135:** 499–505.

GORER, P. A., and Z. B. MIKULSKA, 1954 The antibody response to tumor inoculation. Improved methods of antibody detection. Cancer Research **14:** 651–655.

GRÜNEBERG, H., 1952 The genetics of the mouse. The Hague: Martinus Nijhoff.

HAUSCHKA, T. S., 1952 Immunologic aspects of cancer: A review. Cancer Research **12:** 615–633.

HAUSCHKA, T. S., and A. LEVAN, 1953 Inverse relationship between chromosome ploidy and host-specificity of sixteen transplantable tumors. Exp. Cell Research **4:** 457–467.

HOECKER, G., S. COUNCE, and P. M. SMITH, 1954 The antigens determined by the *H-2* locus: a Rhesus-like system in the mouse. Proc. Nat. Acad. Sci. U. S. **40:** 1040–1051.

LEVAN, A., and T. S. HAUSCHKA, 1953 Endomitotic reduplication mechanisms in ascites tumors of the mouse. J. Nat. Cancer Inst. **14:** 1–44.

LEWIS, E. B., 1951 Pseudoallelism and gene evolution. Cold Spring Harbor Symposia Quant. Biol. **16:** 159–174.

LITTLE, C. C., 1941 The Genetics of Tumor Transplantation. *Biology of the Laboratory Mouse.* Edited by G. D. Snell. Philadelphia: The Blakiston Company.

1947 The genetics of cancer in mice. Biol. Rev. **22:** 315–343.

SCHREK, R., 1936 A biological method for sterilizing contaminated transplantable rat tumors. Am. J. Pathol. **12:** 531–543.

SNELL, G. D., 1948 Methods for the study of histocompatibility genes. J. Genet. **49:** 87–108.

1951a Five alleles at the histocompatibility-2 locus in the mouse as determined by tumor transplantation. Cancer Research **11:** 281–282.

1951b A fifth allele at the histocompatibility-2 locus of the mouse as determined by tumor transplantation. J. Nat. Cancer Inst. **11:** 1299–1305.

1952 Preliminary data on crossing over between *H-2* and *Fu*, *Ki* and *T* in the mouse. Heredity **6:** 247–254.

1953a A cytosieve permitting sterile preparation of suspensions of tumor cells for transplantation. J. Nat. Cancer Inst. **13:** 1511–1515.

1953b The genetics of transplantation. J. Nat. Cancer Inst. **14:** 691–700.

1953c Transplantable Tumors. *The Physiopathology of Cancer.* Edited by F. Homburger and W. H. Fishman. New York: Paul B. Hoeber, Inc.

SNELL, G. D., P. R. F. BORGES, D. E. KELTON, and SALLY LYMAN ALLEN, 1952 Histocompatibility genes in the mouse; methods of study, and some results. Genetics **37:** 626–627.

SNELL, G. D., and G. F. HIGGINS, 1951 Alleles at the histocompatibility-2 locus in the mouse as determined by tumor transplantation. Genetics **36:** 306–310.

SNELL, G. D., E. RUSSELL, E. FEKETE, and P. SMITH, 1953 Resistance of various inbred strains of mice to tumor homoiotransplants, and its relation to the *H-2* allele which they carry. J. Nat. Cancer Inst. **14:** 485–491.

SNELL, G. D., P. SMITH, and F. GABRIELSON, 1953 Analysis of the histocompatibility-2 locus in the mouse. J. Nat. Cancer Inst. **14:** 457–480.

IRRADIATION AND INTERSPECIFIC HYBRIDIZATION IN SECALE[1]

SAM PRICE

U.S.D.A. Agricultural Research Service, Plant Industry Station, Beltsville, Maryland

Received January 28, 1955

HYBRID sterility does not seriously limit genetic exchange between the species, *Secale cereale* L. and *S. montanum* Guss. F_1 hybrids are vigorous and partially fertile. Though these are taxonomically distinct species, the reproductive barriers which separate them are poorly developed. Such species and their partially sterile hybrids provide excellent material for studying the nature and development of hybrid sterility. This is a study of the cytology and partial sterility of F_1 hybrids produced by crossing *S. cereale* and *S. montanum* using both X-rayed and untreated pollen. The observations are discussed in respect to their bearing on the phenomenon of interspecific hybrid sterility.

Hybrids of *Secale cereale* and *S. montanum* have been reported by TSCHERMAK (1927), LONGLEY and SANDO (1930), KOSTOFF (1932, 1937), DUKA (1935), SMITH (1942), and SCHIEMANN and NÜRNBERG-KRÜGER (1952). TSCHERMAK described the hybrids as moderately fertile, as did SMITH. KOSTOFF found 75 percent aborted pollen. DUKA reported pollen viability ranges between five and 38.3 percent; some plants set a small amount of seed while others did not. SCHIEMANN and NÜRNBERG-KRÜGER found 57.65 percent pollen fertility and 37.23 percent seed set in *S. montanum* × *cereale* hybrids and 56.68 percent pollen fertility and 28.75 percent seed set in reciprocals. In their descriptions of meiosis, LONGLEY and SANDO, KOSTOFF, and DUKA report only bivalents and variable frequencies of univalents. SCHIEMANN and NÜRNBERG-KRÜGER describe a maximum association of four bivalents and a translocation chain of six chromosomes. The translocation configuration frequently occurs in lesser associations, such as a chain of four and a bivalent or a chain of four and two univalents.

MATERIALS AND METHODS

Secale cereale L. var. Merced is a variety of cereal rye commonly cultivated in California. It is an erect annual with a continuous rachis, dense spikes, and broad glaucous leaves. *S. montanum* Guss. is a perennial grass native in southwestern Asia. It has narrow leaves, a rachis which disarticulates quite readily, and it flowers later than Merced rye in Berkeley. Two strains of this species were used. The first was obtained from the Copenhagen Botanical Garden; the location of the original collection is not known. This "Copenhagen" strain has slender culms, a slightly spreading habit, and bright green foliage with little glaucousness. The second strain of *S. montanum* was collected in Iran in 1948 by H. P. OLMO. The "Iran" strain resembles cereal rye in that it is erect and has glaucous leaves and culms. Both species are diploid ($n = 7$) and are self incompatible.

[1] Material extracted from a thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Genetics, University of California.

Hybrids obtained from untreated pollinations, hereafter referred to as "control" hybrids, were produced by enclosing emasculated spikes in a paper towel wrapper with one or more spikes of the pollen parent. The method is described in detail by STEBBINS and TOBGY (1944). Hybrids obtained from X-rayed pollinations, hereafter called "r-hybrids", were produced by enclosing irradiated spikes of the male parent in a paper towel wrapper with an emasculated spike of the female parent. A Coolidge X-ray tube operating at three milliamperes and 100 kilovolts was used in irradiating pollen. Culms of the pollen parent were cut when mature pollen was being shed and the spikes were placed fourteen cm from the target. With two ordinary index cards used as filters, the centers of these spikes received approximately 1250r.

The hybrids appear to be intermediate in many of their characters. However, they are mostly erect, have broad leaves, and are taller than either parent. They are extremely variable in their flowering date, some flowering as early as Merced rye. A few did not flower during the first growing season. Plants grown in the field tend to be perennial in Berkeley. Those that did not flower during the first season flowered in the second. The hybrids, Merced rye × Copenhagen *S. montanum* and their reciprocals, have bright green foliage, while Merced rye × Iran *S. montanum* and their reciprocals are glaucous.

Pollen was taken for fertility counts soon after the first spike began to exsert its anthers. The pollen was stained in cotton blue. Those grains which were filled with starch and stained dark blue were considered functional. At least two hundred pollen grains were scored for each plant, except in the case of plants with aborted anthers which contained only shriveled pollen. In determining the percentage of seed set, not less than fifty florets were examined per plant.

Spikelets were fixed in 1:3 acetic alcohol and were stored in 70 percent alcohol. Pollen mother cells were smeared in iron-aceto-carmine. Observations were made on smears permanized by a modification of the alcohol vapor chamber method described by BRADLEY (1948). In each plant 50 metaphase I cells, 100 anaphase I cells, 100 anaphase II half-quartets, and 100 sporads were studied if possible. Where these numbers were not obtainable, fewer cells were accepted.

In testing the significance of differences and the significance of correlations, where variances and covariances are calculated by the product moment method, accurate statements of probability can be made only if the sample is drawn from a normally distributed population, or if the data can be transformed to fit a normal distribution. The data presented here are not normal, nor can they be transformed to fit a normal curve. To avoid the assumption of normality, ranking methods are employed for statistical analyses. Although ranking is an inefficient use of data, this method provides tests about which definite probabilities can be stated. The test of significance of differences used here was devised by WILCOXON (1945) and modified by MANN and WHITNEY (1947). A correction for tied values was given by HEMELRIJK (1952). For determining correlations, the rank correlation coefficient, *Tau*, was computed as described by KENDALL (1948). *Tau*, like the better known correlation coefficient, r, ranges from -1 to $+1$. KENDALL also described the method of testing if *Tau* differs significantly from zero.

RESULTS

Meiosis and fertility in parental strains

Meiosis was studied in five plants of each of the parental strains (table 1). Ten *Secale cereale*, five Copenhagen, and seven Iran *S. montanum* plants were available for fertility studies.

Irregularities in meiosis occur at low frequencies in *S. cereale*. A high frequency of laggards was found in one plant. Inversion bridges and fragments occurred in this and one other plant. Plants which exhibited no other irregularities produced a small percentage of microspores which contained micronuclei. In ten plants of Merced rye the mean pollen fertility was 71.0 percent and ranged from zero to 92 percent. Mean seed set was 71.7 percent and ranged between 56 and 86 percent.

Meiotic irregularities in the strain of *S. montanum* from Copenhagen occurred in low frequencies, but were found in every plant. A few univalents were observed in four of the five. An inversion bridge was found at anaphase I in one plant, and an anaphase II bridge was found in another. Laggards were seen in the second division in two plants, and micronuclei were found in the microspores of all five plants. In this strain the mean pollen fertility was 80.5 percent and ranged from 72.3 to 88.1 percent. The mean seed set was 22.0 percent and ranged from eight to 34 percent.

Two of the five Iran *S. montanum* plants had serious meiotic disturbances. In one the first division proceeded quite regularly, but the second anaphase was characterized by extreme stickiness. The other irregular plant exhibited a high frequency of lagging chromosomes at anaphase I and bridges at anaphase II. Meiosis in the remaining three plants was generally normal but with low frequencies of irregularities. This Iran strain had 64.7 percent fertile pollen. The range in pollen fertility was zero to 96.4. The mean seed set was 33.7 percent with a range of two to 60 percent.

Meiosis in control hybrids

Meiosis was studied in a random sample of ten Merced *Secale cereale* × Copenhagen *S. montanum* and reciprocal hybrids. Ten randomly chosen Merced *S. cereale* × Iran *S. montanum* were also studied. Metaphase I, anaphase I, anaphase II, and sporad stages were observed in detail. Summaries of meiosis in these control hybrids are given in table 1.

Copenhagen vs. Iran hybrids

Ranking analyses were used to compare meiosis in the Merced *S. cereale* × Iran *S. montanum* hybrids with meiosis in hybrids produced in crosses between Merced *S. cereale* and Copenhagen *S. montanum*. The following were used as indices of meiotic chromosome behavior: (1) univalent frequency; (2) chiasmata per cell; (3) percentage of alternately arranged translocation configurations; (4) frequency of anaphase I cells with bridges; (5) frequency of cells with laggards at anaphase I; (6) frequency of second anaphase half-quartets with bridges; (7) frequency of anaphase II half-quartets with laggards; and (8) microspore frequency. Although they involved different strains of the *S. montanum* parent, these two samples of the interspecific hybrids

TABLE 1

Mean observations on meiosis in parental strains and control hybrids

	No. of plants	Metaphase I								Anaphase I			Anaphase II			Micro-spores
		Associations (per cell)						Xmata per cell	Percent alt. trans.	Percent cells with:			Percent half-quartets with:		Percent n = 7 nuclei	Percent with micro-nuclei
		I	II	III	IV	V	IV			bridges	lags.	Unequal distrib.	bridges	lags.		
S. cereale var. Merced	5	0.21	6.89					14.4		1.9	1.2	0.4	0	2.4	96.8	1.1
S. montanum, Copenhagen	5	0.24	6.88					11.9		0.4	2.8	0.8	2.8	3.2	95.6	1.7
S. montanum, Iran	5	0.06	6.97					13.3		1.6	3.6	0.4	2.9*	2.4*	76.2	1.1
Copenhagen control hybrids	10	0.44	4.45	0.14	0.21	0.16	0.43	12.6	61.6	0.9	15.6	15.1	0.2	19.6	66.0	8.2
Iran control hybrids	10	0.24	4.48	0.14	0.24	0.10	0.49	11.9	66.0	0.6	10.4	20.0	0.3	14.1	69.0	7.0
Pollen-sterile control hybrids	14	0.38	4.38	0.10	0.19	0.19	0.46	12.2	62.7	1.2	14.7	13.5	0.6†	16.1†	65.3†	10.3

*Mean of 4 plants.
†Mean of 13 plants.

did not differ significantly ($P > .05$) from each other in any of the eight meiotic characteristics.

The representative sample: control hybrids

Since the two samples of control hybrids did not differ in the comparisons above, they are considered together as a single representative sample in all further comparisons and descriptions. The most outstanding feature of the first metaphase in these control hybrids was the presence of a translocation configuration. The maximum number of chromosomes associated in the configuration was six (figs. 1 and 2), and this maximum was found in several cells in every control hybrid. Chains of three (fig. 3), four, and five (fig. 4) were observed, and occasional cells were seen with associations no greater than bivalents. The translocation configurations were usually open chains. "Frying pan" and Y-shaped associations were found infrequently, but closed rings were not seen.

Good pairing in these hybrids was indicated by low frequencies of univalents. In

FIGURE 1.—Diplotene in control hybrid 178-23, four bivalents and a translocation chain of six chromosomes. × 1100.

FIGURE 2.—Metaphase I in control hybrid 176-6, four bivalents and an alternate translocation chain of six chromosomes. × 1100.

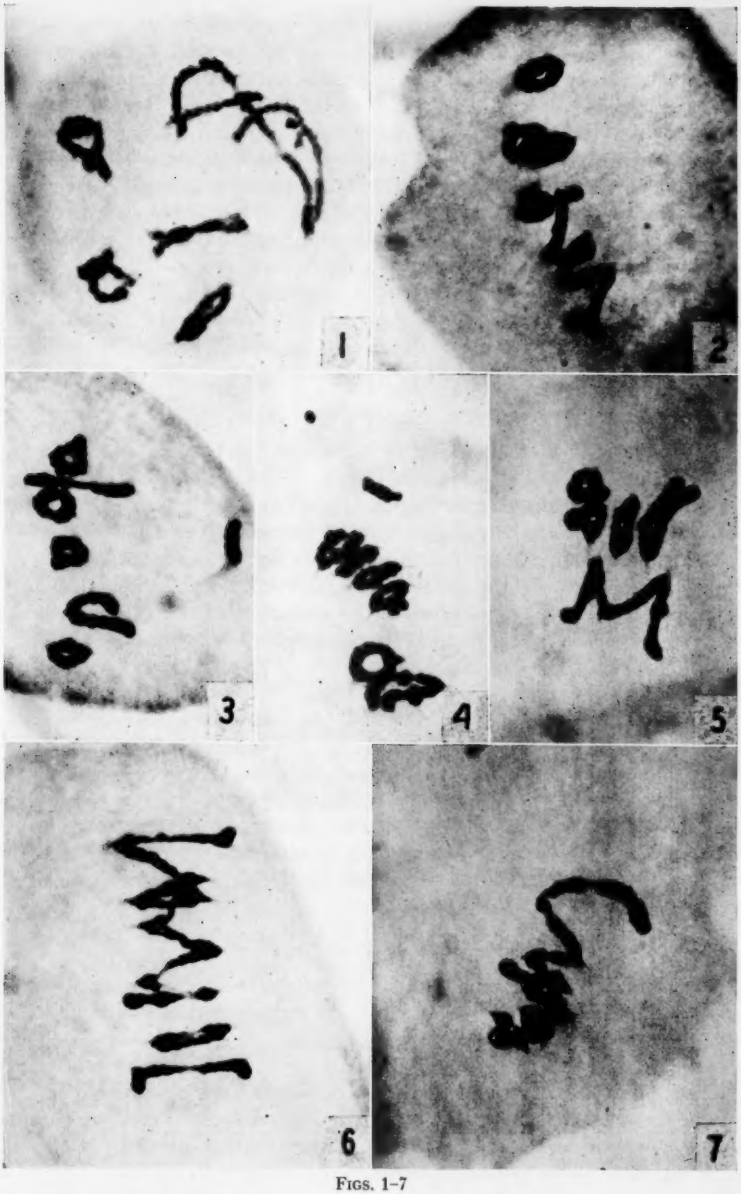
FIGURE 3.—Metaphase I in control hybrid 176-6, a univalent, five bivalents, and a translocation chain of three chromosomes. × 1100.

FIGURE 4.—Metaphase I in pollen sterile control hybrid 180-3, a fragment, one univalent, four bivalents, and a translocation chain of five chromosomes. × 1100.

FIGURE 5.—Metaphase I in r-hybrid 174B-1, three bivalents, a translocation chain of three, and a translocation chain of five chromosomes. × 1100.

FIGURE 6.—Metaphase I in r-hybrid 171-1, three bivalents and an alternate translocation chain of eight chromosomes. × 1100.

FIGURE 7.—Metaphase I in r-hybrid 172-14, three bivalents and an adjacent translocation chain of eight chromosomes. × 1100.



FIGS. 1–7

metaphase I cells there was a close correspondence between high univalent frequency and incomplete six chromosome chains suggesting that most univalents were chromosomes that should have formed a part of the translocation configuration.

Inversion bridges occurred in control hybrids, but in no greater frequencies than in the parental strains. Univalents of metaphase I appeared as laggards at anaphase I. They divided in either the first or the second anaphase. As a result of adjacent disjunction of translocation chains and irregular univalent behavior, unequal numbers of chromosomes were distributed to the poles in about seventeen percent of the first anaphase cells. Distribution in these irregular cells was usually 6:8, but rarely five chromosomes moved to one pole and nine to the other.

Occasional bridges were encountered at anaphase II. Approximately seventeen percent of the half-quartets contained laggards, and only about 67 percent of the nuclei received exactly seven chromosomes in the second division. Micronuclei occurred in about eight percent of the microspores.

Pollen sterile control hybrids

Twenty-five of the 91 control hybrids that flowered in the first season were pollen sterile. Meiosis was studied in fourteen of these (table 1). Taking as meiotic indices the eight characteristics of meiosis used in the test described above, these pollen sterile control hybrids were compared with the representative sample. No significant differences were found ($P > .05$). However, one pollen sterile control hybrid exhibited a fragment in eight of the 50 metaphase I cells examined (fig. 4). With this single exception it may be concluded that no more meiotic irregularities occurred in pollen sterile control hybrids than in the representative sample.

Meiosis in r-hybrids

Two r-hybrids from the cross, Merced rye × Copenhagen *Secale montanum* (r) and four from the reciprocal cross, Copenhagen *S. montanum* × Merced rye (r) were available for studies of meiosis. Random samples of ten Merced rye × Iran *S. montanum* (r) and of ten Iran *S. montanum* × Merced rye (r) hybrids were also studied. Since no differences were demonstrated between control hybrids which involved different strains of *S. montanum*, and since no reciprocal differences were evident, the 26 r-hybrids are considered as a single representative sample. Summaries of meiosis in these r-hybrids are given in table 2.

The representative sample: r-hybrids

The most frequently observed chromosome alterations observed in the r-hybrids were additional reciprocal translocations. First metaphase cells with more than six chromosomes involved in translocation configurations (figs. 5, 6, 7, 8, and 10) provide evidence that new translocations have been induced. Newly induced translocations were found in seventeen of the 26 r-hybrids. In only one of these, No. 168-1, was the induced translocation independent of the original translocation configuration found in control hybrids. Evidence for this induced alteration appeared in only one cell in which the chromosome association was $1_I + 2_{II} + 1_{IV} + 1_V$ (fig. 8).

One plant, No. 171-2, contained two induced translocations which involved at

TABLE 2

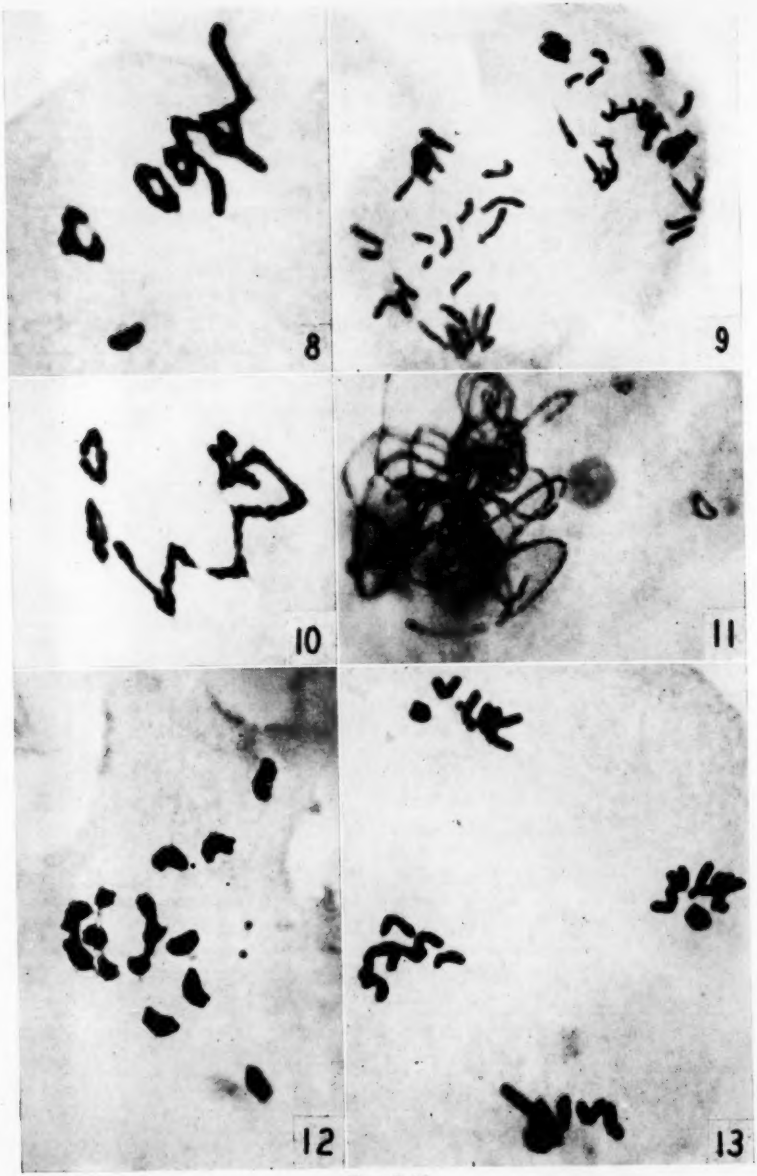
Meiosis in r-hybrids

Plant no.	Fertilities		Metaphase I												Anaphase I			Anaphase II			Micro-spores
			Associations (per cell)										Xmata per cell	Percent alt. trans.	Percent cells with:			Percent half-quartets with:		Percent n = 7 nuclei	Percent with micro-nuclei
	Percent pollen fert.	Percent seed set	I	II	III	IV	V	VI	VII	VIII	IX	X			bridges	lags.	unequal distrib.	bridges	lags.		
165-4	0	68.0	0.18	4.96	0.08	0.66	0.06	0.02					11.3	80.4	1.1	6.7	4.4	0	23.6	55.9	7.0
166-1	0	16.0	0.50	5.14	0.18	0.38	0.04	0.16					12.8	50.0	0.8	5.3	7.1	3.0	10.0	77.0	12.6
166-2	0	34.0	0.28	5.00	0.06	0.26	0.10	0.20					11.4	85.3	0.9	2.8	2.8	0	8.0	90.0	3.8
166-3*	0	4.0	4.12	3.64	0.41	0.24	0.02	0.02		0.02			6.0	74.3	2.6	26.3	34.2	1.0	51.0	25.0	11.9
166-4	0	0	1.08	5.42		0.02							9.3	100.0	0	31.7	15.3	—	—	—	6.8
166-5*	0	0	0.28	5.02	0.25	0.32	0.02	0.18		0.04			10.8	86.8	9.9	6.9	7.9	0	51.7	46.5	13.6
166-6	0	4.0	0.38	4.86	0.16	0.42	0.10	0.20					11.2	75.0	4.4	7.9	7.0	1.8	22.2	75.9	6.2
167-2*	0	54.0	0.56	4.52	0.20	0.36	0.16	0.26					11.0	83.6	0	20.1	9.6	0	2.2	87.7	3.1
167-4*	0	0	0.92	3.54	0.34	0.48	0.20	0.12	0.06	0.12			11.4	32.6	0	4.2	21.3	0	6.8	64.8	5.4
168-1*	5.5	0	0.16	4.78	0.02	0.38	0.06	0.40					11.1	76.7	6.3	5.4	7.2	1.0	7.0	94.0	6.8
170B-1*	0	0	2.20	4.14	0.50	0.30	0.14	0.02					8.4	80.8	20.3	—	—	Fragmentation			32.5
170B-3*	48.8	28.0	0.08	4.10	0.14	0.16	0.06	0.62		0.08			11.9	60.3	4.7	9.5	23.8	2.0	4.0	82.0	6.6
171-2*	9.8	10.0	0.28	2.60	0.14	0.22	0.04	0.14	0.10	0.24	0.04	0.32	11.6	43.3	1.9	7.7	35.9	1.7	10.7	64.2	5.8
171-3	29.5	32.0	0.20	4.28	0.08	0.19	0.16	0.58					12.2	72.0	0.9	5.5	18.3	0	11.0	85.0	4.2
172-3*	0	0	0.04	3.60		0.13		0.34	0.04	0.47			11.6	56.5	0	1.6	32.7	0	6.5	64.2	1.2
172-6*	0	0	0.96	2.64	0.10	0.56	0.02	0.26	0.08	†			10.0	72.0	1.7	—	—	0	—	39.7	29.4
172-7*	11.1	10.0	0.02	3.14	0.04	0.06		0.08	0.02	0.84			13.9	25.0	0	11.9	37.3	0	0	88.0	4.4
172-11*	26.8	10.0	0.06	3.76	0.06	1.56							12.7	88.7	0	0.9	3.9	0.9	5.7	75.0	3.4
172-14*	2.4	13.5	0.01	3.33	0.12	0.12	0.08	0.22	0.06	0.54			12.4	59.6	2.8	6.6	30.1	0	0	74.3	2.2
172-15*	13.5	6.0	0.10	3.34	0.16	0.16	0.08	0.18	0.02	0.56			12.3	58.6	0	5.0	27.0	0	0	84.3	5.0
174A-1*	5.0	18.0	0.90	4.08	0.44	0.24	0.20	0.16	0.02	‡			10.2	79.3	26.9	29.2	13.8	0	45.1	37.0	24.7
174B-1*	1.0	2.0	1.04	3.54	0.16	0.12	0.10	0.12	0.46	0.06			11.2	58.0	1.6	42.6	18.8	—	—	—	7.0
182-1*	12.5	0	0.46	3.82	0.30	0.22	0.10	0.20	0.14	0.18			10.4	57.8	3.7	19.3	19.6	2.7	34.3	40.2	4.7
183-1	43.0	20.0	0.30	4.24	0.26	0.12	0.12	0.56					11.5	69.8	2.7	7.3	26.6	0	26.1	61.5	4.9
184-1	39.5	16.0	0.84	4.44	0.16	0.12	0.28	0.38					12.1	59.5	2.8	1.9	3.8	0	1.9	87.2	7.2
184-2	43.0	20.0	0.88	4.18	0.10	0.32	0.30	0.28					11.7	62.0	6.7	18.2	2.8	0	38.0	29.5	19.6

* Plants which exhibit induced translocations.

† 0.06 Cent. frag.

‡ One frag.



FIGS. 8–13

least one chromosome of the original chain of six and extended the configuration to a maximum of ten chromosomes (fig. 10). Fifteen r-hybrids contained one newly induced chromosome exchange which also involved chromosomes of the original translocation configuration. In one of these, No. 172-11, no configuration of more than four chromosomes was seen. However, many cells had associations of $3_{II} + 2_{IV}$. The newly induced exchange involved the chromosomes of the original chain of six but in such a way that the maximum association of $3_{II} + 1_{VIII}$ was not obtained.

Plants 166-5 and 174A-1 had high frequencies of inversion bridges as well as an induced translocation. Plant 166-3 may have also contained an induced inversion in addition to an induced chromosome exchange. Despite fewer chiasmata, this r-hybrid maintained an inversion bridge frequency comparable to hybrids which had better pairing.

Plant 170B-1 contained an induced translocation and exhibited fragmentation in first and second anaphase (fig. 9). Many bridges were encountered in both anaphase stages, but these were not inversion bridges. Accurate observation of fragmentation was possible in 44 anaphase II cells. Fragmentation was lacking in only five of these; a range of one to fourteen fragments was found in the remaining 39 cells.

In addition to an induced translocation, r-hybrid 172-6 contained a small centric ring fragment which was all that remained of one of the chromosomes. Thirteen chromosomes plus this centric fragment (dividing) can be seen in figure 12. At pachytene the fragment was seen to be a ring (fig. 11). That it contained a centromere was shown by its behavior which was very much like the behavior of univalent chromosomes. Although it sometimes lagged behind, it usually divided at first anaphase (fig. 12). In anaphase II the daughter fragments moved to the poles with the chromosomes or lagged behind. In some cells they appeared to take the place of the missing chromosome, being distributed to the poles that received only six whole chromosomes (fig. 13). A high frequency of micronuclei suggested that the fragment was often left out of telophase II nuclei.

Nine of the 26 r-hybrids lacked evidence of induced translocations. In one of these, No. 166-4, bivalent pairing suggested more chromosome homology than was found in the control hybrids. A translocation configuration was seen in only one cell; this was a chain of four chromosomes. Although the univalent frequency was high (1.08 per cell) and the chiasma frequency was correspondingly low (9.32 per cell), 32 of 50 metaphase I cells contained seven bivalents. One or more structural alterations among the six chromosomes of the original translocation configuration could be postulated to explain the meiotic behavior in this plant. Induced retranslocations

---

FIGURE 8.—Metaphase I in r-hybrid 168-1, a univalent, two bivalents, a "frying pan" translocation configuration of four, and a translocation chain of five chromosomes. × 1100.

FIGURE 9.—Chromosome fragmentation at second anaphase in r-hybrid 170B-1. × 1100.

FIGURE 10.—Metaphase I in r-hybrid 171-2, two bivalents and a translocation configuration of ten chromosomes. × 1100.

FIGURE 11.—Centric ring fragment at pachytene in r-hybrid 172-6. × 1100.

FIGURE 12.—Centric ring fragment dividing at first anaphase in r-hybrid 172-6. × 1100.

FIGURE 13.—Regular distribution of centric fragments at second anaphase in r-hybrid 172-6. × 1100.

would provide a convenient explanation, but inversions could be responsible if they seriously disturbed pairing in the chromosome arms in which they occurred.

Although none of the remaining eight r-hybrids exhibited newly induced translocations, and none of them exceeded the range of control hybrids in the frequency of inversion bridges, slight effects upon meiosis were suggested. Plants 165-4 and 166-1 had low frequencies of chains of six and high frequencies of bivalents. The chiasma frequency (11.22 per cell) in r-hybrid 166-2 was below the range in control hybrids. In this same plant unequal chromosome distribution in anaphase I was less frequent than in any control. In plant 183-1 the frequency of unequal distribution in this stage exceeded that of any control hybrid. Although these are slight differences, they suggest that mutations, deletions, or minute structural rearrangements may have been induced. These plants were compared as a group with the control hybrids in respect to the same eight meiotic characteristics used in earlier tests. The comparison indicated that significantly more r-hybrids contained inversion bridges than did the controls ($P < .01$). No other significant differences were found.

If a large number of differences are tested, as has been done here, some may be significant due to chance alone. However, it seems more than a fortuitous circumstance that the only significant difference here is in the comparison of inversion bridges, the one difference most likely to have been predicted. A difference as great as this would occur by chance in fewer than one out of a hundred tests. Among 20 randomly chosen control hybrids, only nine showed inversion bridges; of the nine r-hybrids lacking induced translocations, eight showed bridges. Induced inversions surely existed in some of these r-hybrids. The inverted segments must have been very small.

Pollen sterile r-hybrids

Preliminary studies of pollen fertility suggested that the progeny of r-hybridizations were less fertile than those of the controls. This seemed to imply that more induced chromosomal alterations might be found among pollen sterile r-hybrids than among those with functional pollen. If the occurrence of new translocations is taken as a measure of induced chromosomal aberration, this expectation is not realized. Twelve of the 26 r-hybrids studied were pollen sterile. Seven (58.3 percent) of these had induced translocations as compared with seventeen (65.3 percent) in the whole sample of 26.

Fertilities studies

The percentage of stainable pollen and the percentage of florets containing seed were determined for 90 control hybrids and for 41 r-hybrids. The mean fertilities of these and of the parental strains are recorded in table 3.

In control hybrids, mean pollen fertility and mean seed set were 18.6 and 20.4 percent respectively. The r-hybrids were 12.2 percent pollen fertile and set seed in 10.7 percent of their florets. The frequency distributions (table 3) show a wide range of variability in pollen fertility and seed set in both control and r-hybrids. A marked shift toward low fertility is seen in the distributions for r-hybrids. In view of the many chromosome alterations induced in the r-hybrids, this apparent reduction in fertility was not unexpected. However, when the differences between control and

TABLE 3

Pollen fertility and seed set distributions in parental strains, control-, and r-hybrids

	Percent										n	Mean	Range
	0–9.9	10–19.9	20–29.9	30–39.9	40–49.9	50–59.9	60–69.9	70–79.9	80–89.9	90–100			
Secale cereale													
P. F.	1			1				3	3	2	10	71.0	0–92.0
S. S.						1	3	3	3		10	71.7	56.0–86.0
Copen. *S. montanum*													
P. F.								2	3		5	86.5	72.3–88.1
S. S.	1	1	1	2							5	22.0	8.0–34.0
Iran *S. montanum*													
P. F.	1				1		1	2		2	7	64.7	0–96.4
S. S.	1	1	1	1	1		2				7	33.7	2.0–60.0
Control hybrids													
P. F.	33	14	17	16	6	4					90	18.6	0–58.7
S. S.	15	30	27	13	3	1		1			90	20.4	0–74.0
r-hybrids													
P. F.	23	8	4	3	3						41	12.2	0–48.9
S. S.	24	10	3	2		1	1				41	10.7	0–68.0

r-hybrids were subjected to tests of significance, only the difference in seed set was significant ($P < .01$). These results are especially surprising since chromosome abnormalities that can be transmitted through eggs are frequently pollen lethals. The original variation was so great that the effect of irradiation on pollen fertility could not be demonstrated.

To test the possibility of independent seed set and pollen fertility, the rank correlation coefficient, *Tau*, was computed. The correlation between seed set and pollen fertility was not significantly different from zero in the control hybrids ($Tau = 0.110$; $P > .05$). The correlation in r-hybrids was small but significant ($Tau = 0.275$; $P < .05$).

This indicates an induced addition to that part of the genetic variation that was common to both pollen fertility and seed set. Most of the induced mutations that affect fertility, whether genic or chromosomal, had similar influences in both male and female gametophytes.

It is regrettable that the data were not amenable to an analysis of variance to determine if there were fertility differences between hybrid families, and to estimate the environmental portion of the variation in fertility. Nevertheless, means were computed for control hybrid families (table 4). Since many families were small, some of the variation in family means undoubtedly is due to random fluctuations, but if only the families of five or more individuals are considered, the range of fertility remains variable. If environmental differences were the only causes of variation, family means would tend to be equal. The wide range of family mean fertilities sug-

TABLE 4

Control hybrid family mean fertilities ranked in order of pollen fertility

Family	No. of plants	Mean percent pollen fert.	Mean percent seed set
180	5	0.00	20.80
178	23	6.58	14.17
179	11	10.46	21.82
176	11	13.56	26.36
177	8	28.67	19.00
169	26	29.61	23.69
185	2	35.78	18.00
186	2	37.86	11.00
187	1	51.14	28.00
173	1	58.71	18.00

TABLE 5

Rank correlation coefficient (Tau) *values: Correlations of fertility with meiosis in control and r-hybrids*

Variables correlated	*Tau* control hybrids	*Tau* r-hybrids
Pollen fertility with:		
% Irregular met. I	0.005	−0.073
% Irregular ana. I	0.156	0.170
% n = 7 nuclei ana. II	0.191	0.074
% Spores with micronuclei	0.143	0.045
Seed set with:		
% Irregular met. I	−0.288	−0.203
% Irregular ana. I	0.118	−0.128
% n = 7 nuclei ana. II	−0.070	−0.004
% Spores with micronuclei	−0.053	−0.124

gests that much of the variation in control hybrids is genetic rather than environmental.

Since much of the variability in fertility appears to be genetic, it is desirable to know if that part of the variability which independently influences pollen fertility and seed set is genetic also. An indication that part of the independent variation is genetic is found again in a consideration of family fertility means of the control hybrids. It will be noticed that the families in table 4 are listed in order of the mean pollen fertility. If the correlation between mean pollen fertility and mean seed set were absolute, this arrangement would automatically succeed in arranging the values for seed set in the same order. In table 4 mean seed set appears to be independent of pollen fertility.

In earlier sections it was pointed out that no differences in meiotic irregularities were demonstrated when pollen sterile control hybrids were compared with the representative sample of control hybrids, nor were detectable induced translocations more frequent in pollen sterile r-hybrids than in the representative sample of r-hybrids. This suggests a lack of correlation between observable meiotic irregularities and fertility. Rank correlation coefficients were computed for correlations between both

pollen fertility and seed set and each of the four following indices of meiotic irregularity for both control and r-hybrids: (1) percentage of metaphase I cells with univalents and/or adjacent translocation configurations; (2) percentage of anaphase I cells with bridges, laggards, and/or unequal chromosome distribution; (3) percentage of anaphase II nuclei receiving seven chromosomes; and (4) the percentage of microspores which contained micronuclei. None of the correlations differed significantly from zero ($P > .05$). The values of *Tau* are listed in table 5. Since variation in the fertility of control hybrids appears to be largely genetic rather than environmental, this lack of correlation between fertility and chromosome behavior points to genic sterility. Genic sterility is the likely source of independent seed set and pollen fertility.

DISCUSSION

Since X-rays are capable of producing the same kind of structural changes that have differentiated the chromosomes of diverging species, the use of irradiated pollen in interspecific hybridization is comparable to crossing species that are even more distantly related. The significant reduction in seed set and the probable, though not demonstrable, effect on pollen viability, suggests a marked increase in hybrid sterility in the r-hybrids of *Secale cereale* and *S. montanum*. Undoubtedly sterility in some of these r-hybrids is the result of induced deficiencies or even lethal gene mutations, and is not comparable with the sterility of other interspecific hybrids. However, the induced translocations and inversions in these *Secale* hybrids are comparable with those that have been responsible for the repatterning of genes in many species. Their contributions to sterility are derived from the same phenomena that produce chromosomal sterility in many other hybrids. Structural alterations and their role in the reproductive isolation of species are thoroughly discussed by STEBBINS (1950, Ch. III and VI) and by DOBZHANSKY (1951, Ch. VIII). The loss of genetic material and the associated centric ring fragment in one r-hybrid is reminiscent of the losses that reduced the basic chromosome number in *Crepis* (TOBGY 1943; SHERMAN 1946). In another r-hybrid, evidence for structural heterozygosity was found in only one first metaphase cell, and pairing suggests more chromosome homology than is observed in control hybrids. If an induced chromosome rearrangement is responsible, this plant represents a step in the direction of "cryptic" structural hybridity (STEBBINS 1945, 1950).

Since chromosome fragmentation has been observed in inbred strains of *Secale cereale* (LAMM 1936; MÜNTZING and AKDIK 1948), the fragmentation in an r-hybrid reported here may not be a product of irradiation. However, fragmentation in this plant may be fully comparable with that in *Triticum* which is controlled by an X-ray induced recessive mutation (SMITH 1939). Interaction of genotypes was proposed as the cause of fragmentation in the meiotic divisions of the hybrids, *Tradescantia canaliculata* × *T. humilis* (GILES 1940), *Bromus Trinii* × *B. maritimus* (WALTERS 1950), and *B. marginatus* × *B. pseudolaevipes* (WALTERS 1952).

Rearrangement of chromosome segments by irradiation is an effective way to produce the same sort of sterility that is found in so many interspecific hybrids. GERASSIMOVA (1939) has produced a strain of *Crepis tectorum* that differs from the

original strain in that each of its chromosomes has been altered by an induced translocation. The new strain is intra-fertile, but when crossed with the original strain produces hybrids that are only about 30 percent fertile. This demonstration of an artificially produced chromosomal sterility barrier suggests a possible use of irradiation in plant breeding. If an improved variety were to be grown in the proximity of unimproved parental material, as a range grass might be, its desirable qualities would be diluted and lost through interbreeding with the parental strain. If a sterility barrier were induced, the new variety could maintain its integrity.

The *Secale cereale* × *S. montanum* control hybrids studied here exhibit both genic and chromosomal sterility. The lack of correlations between chromosome behavior and fertility suggests that much of the partial sterility is genic and is highly variable. Nevertheless, chromosomal sterility must be present. Genetically unbalanced gametes are produced as a result of about 36 percent adjacent disjunction in translocation configurations. Only about 67 percent of the gametes produced by these hybrids receive the proper number of chromosomes.

The indication that at least part of the genic pollen sterility is independent of the genic sterility that influences seed set is of special interest. This independent variation is unusual in interspecific F_1 hybrids. The writer is unaware of other such instances. However, independent segregation of pollen and seed fertility occurs in the C_2 generation of synthetic allopolyploids of *Elymus glaucus* × *Sitanion jubatum* and *Elymus glaucus* × *Sitanion hystrix* hybrids (STEBBINS and VAARAMA 1954) and in F_4 derivatives of the hybrid, *Paraixeris denticulata* × *Crepidiastrum platyphylum* (ONO 1950).

Since genetic sterility has been demonstrated in *Secale* (MÜNTZING 1946, PUTT 1954), it may be suggested that the partial sterility barrier between *Secale cereale* and *S. montanum* is built of the sterility factors that exist within the species. STEBBINS (1950) points out that even in their most significant characteristic, sterility, interspecific hybrids differ quantitatively rather than qualitatively from hybrids within species. KLINGSTEDT (1939) suggests the possibility that sterility genes which are recessive in intraspecific matings might behave as dominants in interspecific hybrids. With this assumption, individuals of two species, if heterozygous for several recessive genes, would produce sterile hybrids when crossed. DOBZHANSKY (1951) objects to this proposal as a general explanation on the grounds that hybrid sterility of this sort would be extremely variable depending upon the strains of the parental species used. The idea of a constant degree of interspecific hybrid sterility is also expressed by STEBBINS (1950, p. 257), "The distinguishing feature of interspecific hybrid sterility is that it is always present and differs relatively little depending on which individuals of parental species are used for crossing." This generalization may well apply to hybrids between species in which a sterility barrier is well established. However, the barrier between *Secale cereale* and *S. montanum* is only weakly developed, and the F_1 hybrid sterility is extremely variable. The wide range of family means suggests that the degree of sterility does depend on the individuals used as parents. The variable sterility in these hybrids might represent the initial stage of development of a sterility barrier between the species. If so, DOBZHANSKY's objection to schemes such as KLINGSTEDT's is not valid here. The hypothesis that hybrid sterility originates from sterility factors already existing within species should not be disregarded.

DOBZHANSKY's own model of the simplest genetic basis for hybrid sterility can be used as a possible model for this origin of a species barrier. His model assumes complementary genes as the basis for hybrid sterility. Beginning with a fertile population of the genotype, *aabb*, a species with the genotype, *AAbb*, might arise by mutation and gene fixation. A second species might diverge with the genotype, *aaBB*. These species are fertile but the hybrids, *AaBb*, are sterile because of the complementary action of *A* and *B*. Since the mutant genes, *A* and *B*, are concerned with sterility in the hybrid, it does not seem unreasonable to revise this model in the following way. Assume additive action for the genes effecting sterility. The genotypes, *Aabb* and *aaBb* would be partially sterile; *AAbb* and *aaBB*, slightly more sterile, etc. In the transition of *aabb* to *AAbb* and to *aaBB*, the first species would include the genotypes *aabb*, *Aabb*, and *AAbb;* while the second would include *aabb*, *aaBb*, and *aaBB*. The two species would exhibit variable partial sterility, and the fertility of hybrids between them would depend upon the individuals used in crossing. A scheme such as this, but built upon more loci, could explain the variable fertility in *Secale cereale*, in *S. montanum*, and in their F_1 hybrids.

SUMMARY

Secale cereale L. var. Merced and two strains of *S. montanum* were crossed using both X-rayed and untreated pollen. Meiosis in interspecific control hybrids did not differ depending on the strain of *S. montanum* used. At first metaphase the maximum association was a translocation chain of six chromosomes plus four bivalents. Univalents, which occurred in low frequencies, were usually chromosomes that were not included in the translocation configuration. Inversion bridges occurred at frequencies no greater than in the parental species. Lagging univalents divided in the first or in the second division. Meiotic irregularities were no more frequent in pollen sterile control hybrids than in the representative sample of control hybrids. Mean pollen and seed fertilities in control hybrids were 18.6 and 20.4 percent respectively.

Of 26 r-hybrids in which meiosis was studied, seventeen contained detectable induced translocations. In sixteen of these, induced translocations involved chromosomes of the original chain of six. Two or perhaps three of the plants with induced translocations also contained induced inversions. Very small inversions were induced in at least some of the nine plants which lacked gross structural changes. One r-hybrid exhibited fragmentation in meiosis. Another contained a small centric ring fragment and had lost chromosomal material. The frequency of induced gross structural rearrangements was no higher in the pollen sterile r-hybrids than in the sample as a whole. The r-hybrids were 12.2 percent pollen fertile and set seed in 10.7 percent of their florets.

The type of chromosome alterations induced in the r-hybrids were the same as those responsible for rearranging the genes in diverging species. This suggests the possibility of inducing chromosomal sterility barriers to prevent interbreeding between a plant breeder's improved varieties and the parental material. Such a barrier would be advantageous if improved varieties are to be grown in the proximity of parental strains.

The percentage of seed set in the r-hybrids was significantly lower than in the controls. The data suggest a similar reduction in pollen fertility, but the difference

was not significant. The correlation between pollen fertility and seed set was significant in r-hybrids but was not in controls. Correlations between fertility and meiotic irregularities were not significant in controls or in r-hybrids. The original as well as the induced chromosomal sterility was largely obscured in these hybrids by genic and perhaps environmental variation in fertility. That much of this variation was genic was indicated by fertility differences between families and the lack of correlation between chromosome behavior and fertility. Part of the genic variability in pollen fertility appeared to be independent of genic variability in seed set.

It is suggested that the variable partial sterility of the control hybrids represents the incipient stage of development of a sterility barrier between *Secale cereale* and *S. montanum*. Such a barrier could have the same genetic basis as the partial sterility that exists within the species.

ACKNOWLEDGMENTS

The writer wishes to express appreciation to the following persons: DR. G. L. STEBBINS, JR., for suggesting the problem and for advice throughout the investigation as well as for reading the manuscript and suggesting improvements; DR. ELIZABETH L. SCOTT and DR. E. R. DEMPSTER for suggestions regarding statistical treatment of the data.

ADDENDA

Brief accounts of the morphology and fertility of *Secale cereale* × *montanum* hybrids are given in the following papers which came to the attention of the author after the present manuscript was submitted for publication:

ANTONOFF, S., 1936 Beitrag zum zytogenetischen Studium der Artbastarde *Triticum turgidum* × *Triticum durum*, *Triticum durum* × *Triticum monococcum* und *Secale cereale* × *Secale montanum*. Der Züchter **8:** 240–243.

NAKAJIMA, G., 1954 Cytogenetic studies on the interspecific hybrids among genus *Secale*. I. Results of hybridization and external characters of F_1 plants. (In Japanese with English summary.) Japan. J. Breeding **4:** 132–134.

LITERATURE CITED

BRADLEY, M. V., 1948 A method of making aceto-carmine squashes permanent without removal of the cover slip. Stain Technol. **23:** 41–44.

DOBZHANSKY, TH., 1951 *Genetics and the Origin of Species*, 3rd ed. Revised, 364 pp. New York: Columbia Univ. Press.

DUKA, S. K., 1935 Cytogenetic research on the interspecific hybrids *Secale vulgare* × *Secale montanum*. Bull. Appl. Bot., Genet., and Plant Breed. Ser. A. **14:** 233–238 (Plant Breed. Abst. **6:** 869).

GERASSIMOVA, H., 1939 Chromosome alterations as a factor of divergence of forms. I. New experimentally produced strains of *C. tectorum* which are physiologically isolated from the original forms owing to reciprocal translocation. Compt. Rend. Acad. Sci. U.R.S.S. **25:** 148–154.

GILES, N., 1940 Spontaneous chromosome aberrations in Tradescantia. Genetics **25:** 69–87.

HEMELRIJK, J., 1952 Note on Wilcoxon's two-sample test when ties are present. Ann. Math. Stat. **23:** 133–135.

KENDALL, M. G., 1948 *Rank Correlation Methods*. 160 pp. London: Chas. Griffin Co.

KLINGSTEDT, H., 1939 Taxonomic and cytological studies on grasshopper hybrids. I. Morphology and spermatogenesis of *Chorthippus bicolor* Carp. × *Ch. biguttulus* L. J. Genet. **37:** 389–420.

KOSTOFF, D., 1932 Pollen abortion in species hybrids. Cytologia **3:** 337–339.

1937 Interspecific hybrids in Secale (rye). I. *Secale cereale* × *Secale ancestrale*, *Secale cereale* × *Secale vavilovii*, *Secale cereale* × *Secale montanum*, and *Secale ancestrale* × *Secale vavilovii* hybrids. Current Sci. **5:** 583–584, also Compt. Rend. Acad. Sci. U.R.S.S. **14:** 213–214.

LAMM, R., 1936 Cytological studies on inbred rye. Hereditas **22:** 217–240.

LONGLEY, A. E., and W. J. SANDO, 1930 Nuclear divisions in the pollen mother cells of Triticum, Aegilops, and Secale and their hybrids. J. Agr. Research **40:** 638–719.

MANN, H. B., and D. R. WHITNEY, 1947 On a test of whether one or two random variables is stochastically larger than the other. Ann. Math. Stat. **18:** 50–60.

MÜNTZING, A., 1946 Sterility in rye populations. Hereditas **32:** 521–549.

MÜNTZING, A., and S. AKDIK, 1948 Cytological disturbances in the first inbred generations of rye. Hereditas **34:** 485–509.

ONO, H., 1950 Intergeneric hybridization in Chichorieae. VIII. Morphology and fertility of F_1 individuals of *Paraixeris denticulata* × *Crepidiastrum platyphyllum*. Japan. J. Genet. **25:** 167–176.

PUTT, E. D., 1954 Cytogenetic studies in rye. Can. J. Agr. Sci. **34:** 81–119.

SCHIEMANN, E., and U. NÜRNBERG-KRÜGER, 1952 Neue Untersuchungen an *Secale africanum* Stapf. II. *Secale africanum* und seine Bastarde mit *Secale montanum* und *Secale cereale*. Naturwissenschaften **39:** 136–137.

SHERMAN, M., 1946 Karyotype evolution: A cytogenetic study of seven species and six interspecific hybrids in *Crepis*. Univ. Calif. Publ. Botany **18:** 369–408.

SMITH, D. C., 1942 Intergeneric hybridization of cereals and other grasses. J. Agr. Research **64:** 33–47.

SMITH, L., 1939 Mutants and linkage studies in *Triticum monococcum* and *T. aegilopoides*. Missouri Agr. Expt. Sta. Research Bull. **298.**

STEBBINS, G. L., JR., 1945 The cytological analysis of species hybrids. Botan. Rev. **11:** 463–486.

1950 *Variation and Evolution in Plants*, 643 pp. New York: Columbia Univ. Press.

STEBBINS, G. L., JR., and H. A. TOBGY, 1944 The cytogenetics of hybrids in Bromus. I. Hybrids within the section Ceratochloa. Am. J. Botany **31:** 1–11.

STEBBINS, G. L., JR., and A. VAARAMA, 1954 Artificial and natural hybrids in the Gramineae, tribe Hordeae. VII. Hybrids and allopolyploids between *Elymus glaucus* and *Sitanion* spp. Genetics **39:** 378–395.

TOBGY, H. A., 1943 A cytological study of *Crepis fuliginosa*, *C. neglecta*, and their F_1 hybrid, and its bearing on the mechanism of phylogenetic reduction in chromosome number. J. Genet. **45:** 67–111.

TSCHERMAK, E., 1927 Über seltene Getreide- und Rübenbastarde. Zeit. Ind. Abst. Ver. **2:** 1495–1498.

WALTERS, M. S., 1950 Spontaneous breakage and reunion of meiotic chromosomes in the hybrid *Bromus Trinnii* × *B. maritimus*. Genetics **35:** 11–37.

1952 Spontaneous chromosome breakage and atypical chromosome movement in meiosis of the hybrid *Bromus marginatus* × *B. pseudolaevipes*. Genetics **37:** 8–25.

WILCOXON, F., 1945 Individual comparisons by ranking methods. Biometrics **1:** 80–83.

THE GENETIC CONTROL OF MATING TYPE POTENTIALITIES IN TETRAHYMENA PYRIFORMIS[1]

D. L. NANNEY, P. A. CAUGHEY AND A. TEFANKJIAN

Zoology Department, University of Michigan, Ann Arbor

Received February 1, 1955

GENES controlling mating type potentialities in *Paramecium aurelia* (SONNEBORN 1939) were the first mendelizing factors demonstrated in the Ciliates. Although other genes have since been discovered in *P. aurelia*, the mating type genes were of particular significance in that, for the first time, they provided unequivocal evidence for classical genetic mechanisms in the Ciliates and a firm basis for subsequent studies in cellular heredity.

Genes have also been demonstrated in another Ciliate, *Euplotes patella* (KIMBALL 1942), and these include factors concerned with mating type inheritance. The modes of action of the mating type genes in these forms appear to be different. The Paramecium genes confer on the cell containing them a potentiality, the realization of which may depend upon other circumstances; the mating type genes in Euplotes appear directly to determine the mating type of a cell.

MATERIALS AND METHODS

Materials

This report is concerned with evidence for genes controlling mating type potentialities in variety 1 of *Tetrahymena pyriformis*. This species, like several species of Paramecium (SONNEBORN 1947), is composed of a number of non-interbreeding groups designated as "varieties" (GRUCHY 1954). Within each of the varieties from 2 to 8 different mating types have been identified; conjugation occurs, within a variety, when any two clones of different types are combined. At the present time 7 mating types are known for variety 1 (NANNEY and CAUGHEY 1953). The strains used in this study were derived from two clones isolated from a pond near Woods Hole, Massachusetts, by ELLIOTT and GRUCHY (1952) and are designated as the WH strains. All the 7 known mating types have appeared among the progeny of these clones.

Methods

Since the culture methods and techniques used to determine the mating types of unknown cultures are presented elsewhere (NANNEY and CAUGHEY 1955), the general procedures will be indicated only briefly here. Immediately following conjugation the exconjugants and the first fission products are separated into individual containers and allowed to give rise to cultures. These exconjugant cultures are immature and must undergo about 80 fissions before their mating types can be determined.

[1] This study was supported by a grant from the National Science Foundation, by grants from the Faculty Research Fund and by a Faculty Summer Fellowship from the Horace H. Rackham School of Graduate Studies of the University of Michigan.

The procedure for obtaining mature cultures is to make serial isolations from the exconjugant cultures, single animals being isolated—usually at two day intervals—for a total of 8 to 10 transfers. At the end of this time the cultures are placed in test tubes. Finally these mature cultures are mixed with samples of each of the standard mating types and are examined for conjugants. Every culture examined has mated with 6 of the 7 standard types and has been classified as belonging to the type with which it did not mate.

Cytogenetics

The cytological events occurring at conjugation in Tetrahymena have been discussed in detail by NANNEY (1953), by ELLIOTT and HAYES (1953) and by RAY (1954). The cytological events of genetic significance are as follows. Meiosis occurs in each member of a conjugating pair and results in the formation from the single original diploid micronucleus of four haploid nuclei, each with 5 chromosomes. Three of the four haploid nuclei disintegrate and the fourth undergoes an equational division—giving rise to a migratory and a stationary nucleus. An exchange of the migratory nuclei occurs between the two members of a pair and is followed by a fusion of the migratory and stationary nuclei of diverse origin. The cytological events lead to the expectation that pair members, regardless of their original genetic constitutions, will become alike following conjugation. If the original parents are homozygous for different alleles at a particular locus, the exconjugant F_1 will be heterozygous. Segregation will occur when F_1 clones are crossed; one fourth of the F_2 *pairs* will be homozygous for one allele, one half will be heterozygous and one fourth will be homozygous for the other allele.

The fertilization nucleus produced at conjugation undergoes two divisions before the exconjugant divides. Two of the four nuclei so produced become macronuclei and two become micronuclei; one of the micronuclei disintegrates. At the first cell division following conjugation the single remaining micronucleus divides, but the two new macronuclei are separated into different cells to divide at all subsequent divisions. This first cell division is, therefore, unique in that diverse lines of cells (caryonides), deriving their macronuclei from two different primordial macronuclei, are separated at this division. In Tetrahymena (NANNEY and CAUGHEY 1953), as in *P. aurelia* (SONNEBORN 1937), sister caryonides derived from the same exconjugant may show different mating types, but—in general—the cells within a caryonide are of the same mating type. Occasionally clones are found which contain two or more mating types and which conjugate (self) in clonal cultures. These selfing clones are discussed at length elsewhere (NANNEY and CAUGHEY 1955) and only those selfers from which pure types have been derived are utilized in the present analysis. Caryonidal inheritance (SONNEBORN 1947; NANNEY 1954) indicates that the mating types in these forms are controlled by the macronuclei and that diverse mating types may be developed on a common genetic basis. On the other hand, it has been shown for *P. aurelia* that the mating type *potentialities* (SONNEBORN 1947) and the mating type *frequencies* (BUTZEL 1953) are determined in some cases at least by nuclear genes. Evidence presented below demonstrates that the spectrum of possible mating types in Tetrahymena is also controlled by genes.

Tests for conjugation

The two strains on which the present study is based are designated as WH-6 (mating type I) and WH-14 (mating type II). Crosses involving these two strains yield many pairs with cytological irregularities and few viable progeny are produced. It has been possible, however, to derive some apparently normal exconjugant clones from such crosses. Further crosses with these derived strains show relatively little inviability and few cytological anomalies. Nevertheless, occasional pairs are found which separate without forming new macronuclei. Such pairs must be detected and eliminated if a genetic analysis is to be significant. Several tests for nuclear reorganization have been utilized in these experiments. In the earliest crosses, after conjugation, one member of each pair was killed, stained and examined for new macronuclei. Examination of hundreds of pairs stained just prior to separation has revealed no cases in which one member had produced new macronuclei and the other member had not. Hence, it appears reasonable to conclude that if the killed exconjugant has new macronuclei, its living mate has also undergone a nuclear reorganization. This procedure is the most direct method for demonstrating that reorganization has occurred, but it requires the sacrifice of half the information a pair might provide.

The second test for reorganization was derived from an observation made during the experiments described above. Every cell stained and found to contain new macronuclei had been paired with a cell which gave rise to an immature clone, i.e., a clone which would not mate until many fissions later. Conversely, every cell stained and found to contain no new macronuclei had been paired with a cell which gave rise to a clone which was mature as soon as it could be tested. Therefore, an examination of "exconjugant" clones for early maturity constitutes a satisfactory screening procedure for non-conjugants. Approximately half the crosses to be reported were screened in this fashion.

A third test, also based on the initial observations, has been used extensively but has not proved very satisfactory. This test is based on the fact that pairs which do not undergo reorganization do not change mating type; when sub-lines are derived at the first cell division following separation, four clones are derived from a pair—two of one parental mating type from one cell and two of the other parental mating type from its mate. Any pair which produces clones which deviate from this pattern of mating type distribution may be considered to have undergone a nuclear reorganization. Since several different mating types are produced in all crosses and since these are distributed among the caryonides approximately at random, relatively few conjugants will be falsely excluded so long as all four caryonides are available for testing. This is particularly true if one or both of the parental types is rare. The chief difficulty arises from the fact that all four caryonides are not always available for testing; one or more caryonides may die, or may be accidentally lost in making isolations. Moreover, it is not always desirable to obtain all four caryonides when representatives from a maximum number of *pairs* are desired. Under these circumstances some pairs excluded as possible non-conjugants are certainly rejected falsely. These false exclusions result in a distortion of the relative frequencies of the mating types among the progeny—to different degrees and in different directions depending upon the mating types of the parents. In the experiments reported here, the significant data are largely

qualitative and the frequency distortions are not critical. The chief bearing of the excluded clones is upon the total number of progeny used to conclude that a particular mating type *cannot* appear. The exclusion of clones as possible non-conjugants is, therefore, a conservative factor.

None of the above tests for nuclear reorganization adequately distinguishes between true conjugation (involving an exchange of genetic material between pair members) and cytogamy (mutual self fertilization). In certain instances it is clear that an exchange of material has occurred, but no generally valid procedure exists for excluding cytogamous pairs. Evidence will be presented later, however, which indicates that most, perhaps all, of the reorganization is conjugation.

Progeny tests

The studies on caryonidal inheritance in *Paramecium aurelia* (SONNEBORN 1947) demonstrate that clones with the same genotype may possess different mating types. Moreover, clones with the same mating type may have different genotypes. As will be shown, a similar situation occurs with Tetrahymena. Under such circumstances the "phenotype" governed by a particular genotype is not adequately manifested by any one clone. The genotype determines a spectrum of possibilities which can be discerned only by examining large numbers of clones. Whenever possible these clones have been obtained through the use of progeny tests in which sister caryonides of different mating types are crossed.

Crosses between sister caryonides should yield progeny exclusively of the parental genotype only if the parents are homozygous. If the parents are heterozygous for one or more factors a variety of genotypes would be expected among the progeny, and the parental genotype might be rare. Hence, if certain mating type potentialities are determined by heterozygous genotypes, a parental potentiality might be lost through recombination, or a potentiality not possessed by the parental genotypes might appear among the progeny. Fortunately, this complication has not arisen under conditions suitable for its detection. Specifically, heterozygous determination of a potentiality would be detected in a cross of diverse inbred strains by the appearance of a mating type which neither inbred strain alone could produce. Such novel types have not been detected.

In certain instances, it is either impossible to cross sister caryonides or to examine their progeny. Under these circumstances, the clones must be outcrossed to other clones of known potentiality. For example, a clone of unknown potentiality may be crossed to a clone which *cannot* produce mating type I; if mating type I appears among the progeny, one may conclude that the tested clone possessed the potentiality for that type. This outcross procedure has been validated by outcrosses of clones which can also be subjected to analysis by crosses of sister caryonides. The outcross method is much less economical than the sister × sister progeny tests, but it is the only practicable method for classifying some clones.

THE GENETIC ANALYSIS

Two reasonably homogeneous strains have been obtained through inbreeding the F_1 cultures. The first inbred series, designated as "family A", has been carried through

six generations; crosses among the various related clones regularly produce 5 of the 7 mating types known for this variety; only types IV and VII are lacking. The second inbred series, designated as "family B", has been carried for five generations; crosses between clones in this group produce all the known types except type I. Only a fraction of the possible combinations of parental mating types in either family have been examined in the crosses made; only 4 of the 10 possible combinations in family A have been studied and only 4 of the 15 combinations in family B. Since cells of the same mating type could be of different genotype, an exhaustive study of the mating type potentialities within the families would require a very large number of crosses. It is possible, therefore, that some as yet unexamined crosses will yield types other than those thus far observed. On the other hand, the data available (table 1) suggest a reasonable uniformity among the progeny within a family, regardless of the parental mating types. Occasionally some type which should appear is missing from a particular cross. Thus in family A, the cross F_3 II $\times$ VI did not yield type V. Since the types missing are in each case rare types, their absence is probably due to sampling error. Moreover, in some cases the crosses were repeated at different temperatures and the missing types were found. At no temperature, however, were types IV or VII found in family A or type I in family B. That a real genetic difference characterizes these families is further borne out by the subsequent breeding analysis.

The plan for analyzing the genetic basis for the observed differences in mating type potentialities was as follows. A representative of family A was crossed with a representative of family B and the progeny of 20 pairs were tested for mating type. These progeny constituted the F_1 generation. Three of these F_1 pairs were chosen to produce the F_2 by crosses of sister caryonides. The F_2 pairs were then analyzed for mating type potentialities by conducting progeny tests.

The mating types of their progeny indicated that all the 20 F_1 pairs had undergone a nuclear reorganization. Eight of the 20 pairs showed evidence of genetic transfer; in these 8 pairs, either one conjugant produced mating types originally found only in different families (I and IV or I and VII) or both conjugants produced mating types found originally in only one parental family (I and I; IV and IV; VII and VII or IV and VII). More of the pairs would probably have been clearly judged to be true conjugants if all the caryonides had been available for analysis, but 19 of the 80 caryonides either selfed, were accidentally lost or died before reaching maturity. All 7 mating types appeared in the F_1. This fact at once suggests that if the capacities to produce these mating types are under genetic control, and if the parents were homozygous for different mating type potentialities—the genes giving a cell a particular potentiality are expressed in the heterozygous condition.

The F_1 pairs chosen as parents for the F_2 were selected for good viability and a diversity of mating types. Two of the pairs were among the 8 giving evidence of genetic exchange; the third pair had undergone a reorganization, but cytogamy could not be excluded. The F_2 was derived from the following crosses: cross 1—between types II and V from the same member of an F_1 pair (the possibly cytogamous pair), cross 2—between types III and IV, also sister caryonides, and cross 3—between types I and VII from a single member of a third pair. Each of these crosses produced in either the F_2 or a subsequent generation some progeny of each of the 7 mating types. Since

TABLE 1

The distribution of mating types among the progeny of crosses within inbred strains of Tetrahymena pyriformis, *variety 1*

Parents	Mating types of progeny							
	I	II	III	IV	V	VI	VII	Total
A. Family A								
F_1 II × VI	3	1	4	0	2	2	0	12
F_2 I × III	4	6	1	0	10	7	0	28
F_3 II × VI	9	6	9	0	0	3	0	27
F_3 II × V	12	2	4	0	2	4	0	24
F_4 III × VI	17	11	10	0	8	27	0	73
F_4 I × III	9	9	15	0	2	17	0	52
Totals.....	54	35	43	0	24	60	0	216
B. Family B								
F_1 IV × V	0	16	0	29	7	9	5	66
F_1 IV × F_2 VII	0	7	6	31	6	24	12	86
F_2 II × V	0	10	0	9	1	1	5	26
F_3 IV × VII	0	2	1	2	2	8	4	19
F_3 IV × V	0	5	7	28	0	3	4	47
F_4 IV × VI	0	50	21	154	7	19	32	283
Totals.....	0	90	35	253	23	64	62	527

the parents in each cross were derived from a *single* F_1 exconjugant, an exchange of mating type factors must have occurred in the initial cross, at least in these cases. For practical purposes we may assume that both parents (P_1) were homozygous and that the F_1 pairs used were heterozygous for factors differentiating the two families.

Two of the 45 F_2 pairs were excluded from further analysis as probable non-conjugants; all 4 caryonides were available, two of one parental mating type from one member and two of the other parental type from its mate. Six of the F_2 pairs failed to produce progeny viable at the time of maturity. Four of the F_2 pairs produced only clones of an abnormal kind, designated as "semi-amicronucleate". These clones contain large numbers of amicronucleate cells, which are visibly abnormal; the amicronucleates do not multiply indefinitely under our cultural conditions and the clones are perpetuated from the cells having micronuclei. The semi-amicronucleate condition is usually detected only after 40 to 50 fissions and is usually observed in all or none of the caryonides from a pair. Both the normal and the amicronucleate cells in such cultures can conjugate, but the viability of the progeny is extremely low. These clones were, therefore, eliminated from the analysis. Thus, of the 45 original pairs obtained, only 33 were analyzed fully, 11 from each of the 3 crosses.

Progeny tests on the F_2 pairs revealed only 3 obvious classes: *class 1*—lacking the ability to produce types IV and VII, *class 2*—producing all 7 types and *class 3*—lacking the ability to produce type I. The potentialities observed in the P_1, the F_1 and the F_2 are summarized in figure 1. In this figure the mating type frequencies are represented as sectors in a circle. The P_1 frequencies are derived from the accumu-

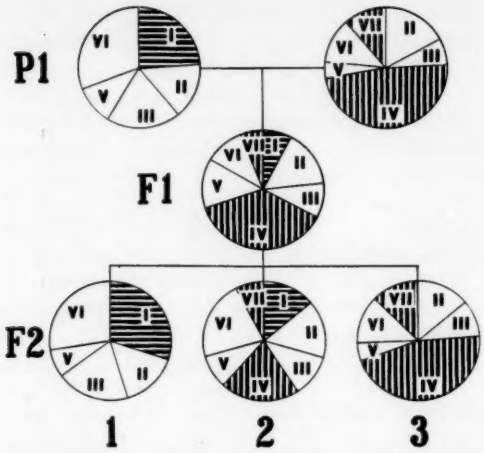


FIGURE 1.—Diagrammatic representation of crosses; mating type frequencies indicated as sectors in a circle. The P_1 frequencies are derived from the accumulated data for families A and B; the F_1 frequencies are derived directly from the F_1; the F_2 frequencies for the three classes are based on progeny tests in which sister caryonides from single F_2 pairs were crossed. The horizontal hatching designates the type found uniquely in family A; the vertical hatching designates the types found uniquely in family B.

lated data for families A and B. The F_1 frequencies are derived from the 20 F_1 pairs examined. The F_2 frequencies are the average frequencies observed for each class when crossed to sister caryonides; the outcross data are not considered. Class 1 in the F_2 resembles family A both in the kinds and the frequencies of mating types; class 3 closely resembles family B.

The results of progeny tests on individual F_2 pairs are summarized in table 2. The data are given only for the 3 critical mating types, but all the pertinent progeny for these types are included, whether produced in the F_2, the F_3, the F_4 or in outcrosses. The confidence limits shown are the 95% confidence limits of the observed frequencies, expressed in percentages. Considerable variation is found in the frequency of a type, even within a class. This is probably due to the fact that some F_2 pairs were analyzed almost exclusively by outcrosses, some almost exclusively by sister × sister crosses and some by both means. If an F_2 pair were heterozygous for a capacity, it should produce different frequencies of a type when crossed with itself and when crossed to a homozygous recessive clone. The data may also be slightly biased by the procedures involved in determining the sizes of the samples. Each F_2 was first studied by an examination of 20 to 30 progeny (caryonides). Later, another sample of similar size was obtained. If these small samples included relatively large numbers of the critical types no further progeny tests were made. If few or none of a particular type appeared, still further samples were added until a reasonably large total was available. Because of this selective increase in sample size, the frequencies of certain types in small samples may be unduly high. Finally, some bias may have resulted from the procedures discussed above in the section on Tests for Conjugation.

TABLE 2

The frequencies of the significant mating types produced in progeny tests of the F_2. The first numbers in the pair designations refer to the three crosses (discussed in the text) from which the F_2 was derived

F_2 pairs	Mating type I			Mating type IV			Mating type VII		
	No.	Total	Conf. lim.**	No.	Total	Conf. lim.**	No.	Total	Conf. lim.**
Class 1									
1–1*	7	43	7–31	0	89	0–4	0	89	0–4
1–6	27	94	20–40	0	94	0–4	0	94	0–4
1–10*	9	77	6–21	0	110	0–3	0	110	0–3
2–9*	17	72	15–35	0	85	0–4	0	85	0–4
3–4*	7	28	11–45	0	63	0–6	0	63	0–6
3–5*	14	86	9–26	0	144	0–3	0	144	0–3
3–10*	8	61	6–14	0	88	0–4	0	88	0–4
3–12*	13	80	10–26	0	80	0–5	0	80	0–5
Class 2									
1–2*	6	98	2–13	16	94	10–26	6	94	2–13
1–3*	4	87	1–11	11	90	6–21	7	90	3–15
1–4	8	41	9–35	10	41	12–40	3	41	2–20
1–7	6	49	5–25	10	49	10–34	3	49	1–17
1–13*	5	81	2–14	7	78	4–18	3	78	1–11
1–14	11	65	9–28	19	65	19–42	7	65	4–21
1–15*	7	45	6–30	7	115	2–12	9	115	4–16
2–1*	8	54	7–27	12	83	8–24	4	83	1–12
2–3	11	74	8–26	11	74	8–25	7	74	4–19
2–6	8	67	5–22	10	67	7–25	2	67	<1–10
2–10	9	51	8–31	19	51	24–52	2	51	<1–13
2–11	13	80	9–26	18	80	14–33	4	80	1–12
2–13	14	95	8–23	8	95	4–16	3	95	1–9
2–14	3	73	1–12	27	73	25–49	9	73	6–22
2–15	7	71	4–19	13	71	10–30	9	71	6–23
3–1*	10	86	6–20	17	115	9–23	8	115	3–13
3–7	12	87	7–23	16	87	11–29	5	87	2–13
3–13	10	66	7–26	19	66	19–42	1	66	<1–8
3–15	10	49	10–34	19	49	26–54	2	49	<1–14
Class 3									
1–11*	0	74	0–5	11	36	16–47	5	36	5–30
2–4	0	80	0–5	38	80	35–58	8	80	4–19
2–7*	0	81	0–4	7	37	8–35	8	37	10–38
3–6	0	98	0–4	40	98	31–51	8	98	4–16
3–8	0	107	0–3	55	107	41–61	14	107	8–21
3–9	0	78	0–5	33	78	31–54	10	78	6–22

* F_2 pairs tested wholly or in part by outcrosses.

** 95% confidence limits expressed in percentages.

In spite of these difficulties in quantitative interpretation, certain general conclusions seem secure. (1.) Certain F_2 pairs (class 3) lack the ability to produce mating type I, while others (classes 1 and 2) produce this type in appreciable numbers. Little overlap is observed in the confidence limits for these two groups. (2.) Certain other F_2 pairs (class 1) lack the ability to produce type IV. Again, little overlap is observed

in the confidence limits for those pairs which did and those which did not produce this type. (3.) Certain F_2 pairs (class 1) produced no mating type VII, but—because type VII is a rare type—the confidence limits of the frequencies for pairs producing this type overlap considerably the limits for pairs failing to produce the type. Nevertheless, it is significant that every pair producing type VII also produced type IV and no pair failing to produce type VII produced any type IV. (4.) Every F_2 pair produced at least one of the three critical types. (5.) The frequencies of type I are generally highest among the progeny of pairs which produced none of types IV and VII. Conversely, the highest frequencies of types IV and VII are found among the progeny of pairs failing to produce type I.

Three interpretations for these data may be considered. The simplest interpretation would attribute the observations to the existence of a pair of alleles, $mt^{(I)-}$ and $mt^{(IV,\ VII)-}$, at the same locus. According to this scheme cells homozygous for the $mt^{(I)-}$ allele would be unable to produce mating type I, but could produce any of the other 6 known mating types; family B and class 3 of the F_2 would have this genotype. Cells homozygous for the other allele, $mt^{(IV,\ VII)-}$, would be unable to produce types IV and VII, but could produce any of the other 5 mating types; family A and class 1 of the F_2 would have this genotype. Finally, the heterozygote would be able to produce any of the 7 known types; the F_1 in the cross between families A and B and class 2 of the F_2 would have this genotype. The F_2 should consist of these three classes in a ratio of 1:1:2; since 33 F_2 pairs were examined, the expected ratios are 8.25:8.25:16.5. This is in satisfactory agreement with the observed ratio, 6:8:19.

According to a second interpretation genes would occur at two independent loci, one locus determining the ability to produce type I and the other determining the ability to produce types IV and VII. A dominant allele at a particular locus would permit the expression of a given type (or types) and the recessive allele would fail to permit that type. Family A would be homozygous for the allele permitting mating type I and also for the allele failing to permit types IV and VII; family B would have the reciprocal homozygous genotype. The F_1 would be heterozygous for both factors. The F_2 would consist of four phenotypic classes, the three observed plus a fourth—corresponding to the double recessive—which would lack the abilities to produce mating types I, IV and VII. The expected phenotypic ratio among the 33 F_2 pairs examined would be 6.2:6.2:18.6:2.1. The observed ratio, 6:8:19:0, does not differ significantly from this expectation.

The distinction between these two hypotheses involves the presence or absence of the F_2 class unable to produce types I, IV and VII. Since the F_2 data were not sufficiently numerous to exclude its existence, further crosses had to be undertaken. The rationale for these crosses was as follows. According to the two-factor hypothesis class 2 of the F_2, which is capable of producing all 7 mating types, should consist of 4 genotypic classes. Only one of these, the double heterozygote, could produce the missing genotype through recombination. This double heterozygote should make up $\frac{4}{9}$ of the class 2 pairs and should have a probability of $\frac{1}{16}$ of producing the double recessive at sexual reorganization. Hence, the probability for a double recessive among the sexual progeny of this F_2 class is ($\frac{4}{9} \times \frac{1}{16}$) or about 0.028.

Similarly, class 3 of the F_2, which is incapable of producing type I, should be com-

TABLE 3

Evidence against the existence of a double recessive genotype

Source	Probability for double recessive on two-factor hypothesis		Total examined	Number	
				Expected	Found
F_2	0.062		33	2.1	0
	Unadjusted	Adjusted			
F_3—Class 1	0.17	0.43	7	2.9	0
Class 2	0.028	0.09	8	0.7	0
Class 3	0.17	0.85	3	2.5	0
Totals			51	8.2	0

* Adjusted for screening procedure described in text.

posed of two genotypic classes, one of which should comprise $\frac{2}{3}$ of the total and have the probability of $\frac{1}{4}$ of producing the double recessive. Therefore, the probability for a double recessive among the sexual progeny of class 3 is ($\frac{2}{3} \times \frac{1}{4}$) or about 0.17. Identical considerations hold for the progeny of class 1.

Although each of these probabilities is low, they may be greatly enhanced by a judicious selection of F_3 pairs for further analysis. For example, among the progeny of class 2, 70% of the pairs with two or more caryonides available showed one or more of the three critical types and could be excluded from consideration. The probability that one of the remaining 30% is a double recessive is thereby increased from 0.028 to about 0.09. Similar considerations hold for the progeny of the other two F_2 classes. 60% of the progeny of class 1 could be excluded, thus raising the probability for a double recessive among the remaining 40% from 0.17 to about 0.42. 80% of the progeny of class 3 could be excluded, thus raising the probability for a double recessive from 0.17 to about 0.85.

These considerations and the results of the analysis of selected F_3 pairs are summarized in table 3. The phenotypic class postulated on the two-factor hypothesis has not appeared under conditions under which it would be reasonably expected. It must be concluded, therefore, that the class does not exist, at least in the frequency demanded by the hypothesis in its simplest form. One reservation should be kept in mind, however. Present data are not sufficiently good to eliminate the possibility that the double recessive has not appeared because it has reduced viability. The hypothesis of two independent loci is, therefore, rejected primarily because it requires more assumptions than the hypothesis of alleles at a single locus.

A third general explanation for these results may also be considered, that two or even three loci on a single chromosome are responsible for the results observed. This hypothesis would be applicable, even if many more data were available, by assuming very close linkage of the various loci involved. In the absence of good screening techniques it will be difficult to render this explanation even improbable. If, on the other hand, progeny attributable to crossing-over eventually appear, this hypothesis will be strongly supported. At the present time, however, the simplest interpretation of the data is that involving alleles at a single locus.

TABLE 4

Evidence for the equivalence of class 1 of the F_2 and family A

Crosses	Mating types					
	I	II	III	V	VI	Total
Family A × Family A	54	35	43	24	60	216
Family A × Class 1	93	63	61	32	117	366
Class 1 × Class 1	58	42	37	21	69	227
Totals	205	140	141	77	246	809

$\chi^2 = 2.4$
d.f. = 8
P = 0.95–0.98

TABLE 5

Evidence for the equivalence of class 3 of the F_2 and family B

Crosses	Mating types						
	II	III	IV	V	VI	VII	Total
Family B × Family B	90	35	253	23	64	62	527
Family B × Class 3	18	14	73	4	26	9	144
Class 3 × Class 3	46	35	156	20	44	38	339
Totals	154	84	482	47	134	109	1010

$\chi^2 = 12.7$
d.f. = 10
P = 0.2–0.3

The single locus hypothesis receives some additional support from certain quantitative considerations. If class 1 is genetically equivalent to family A, crosses of class 1 × class 1, class 1 × family A and family A × family A should yield not only the same mating types among the progeny, but the same frequencies of these mating types. If other factors were involved, this agreement might not be expected. Although, as pointed out above, quantitative treatment of present data is not entirely justified because of possible frequency distortions, attempts to verify this expectation have been made (table 4). The results are in satisfactory agreement with the hypothesis. Similarly a genetic equivalence of class 3 and family B is suggested by the data in table 5.

DISCUSSION

The data presented in the previous section indicate that differences in mating type potentialities in two inbred strains of *Tetrahymena pyriformis* are dependent upon differences in a single chromosome. The strains designated as family A are homozygous for an allele $mt^{(IV,\ VII)-}$; this genotype permits the expression of mating types I, II, III, V and VI in characteristic frequencies. The strains designated as family B are homozygous for the allele $mt^{(I)-}$; this genotype permits the expression of mating types II, III, IV, V, VI and VII. Any of the 7 known types may appear in the hetero-

zygote. Although the available data are consistent with this interpretation, they do not rule out the possibility that the *mt* locus consists of two or more linked genes. Because screening techniques for recombinants have not been perfected, it is unlikely that this situation will be clarified soon. It should be possible, however, to find other genes and perhaps other mating types in collections from the wild. Particularly, it is to be anticipated that an 8th mating type will appear, since the similar but more extensively studied multiple mating systems in *Paramecium bursaria* contain 2, 4 or 8 mating types (see METZ 1954 for discussion and references). The information now available for the varieties of *T. pyriformis* (GRUCHY 1954) suggests a similar "numerological" system for this species. If several genes can be obtained for this system, they will be of great value in studying the mechanisms of mating type determination in the Ciliates.

Even the limited information now available on the mating type genes in Tetrahymena is worth considering in a discussion of Ciliate sexuality. According to METZ's (1954) interpretation of the peculiar geometric progression of numbers of mating types, systems of two mating types may be explained on the basis of the presence or absence of a single substance; systems of four mating types may be explained as due to the presence or absence of two substances independent of one another; and systems of eight mating types would require various combinations of three substances. This interpretation suggests that the elimination of one substance in an 8-type system will reduce it to a 4-type system, simultaneously removing four of the mating types. Since it seems reasonable to assume that genes would influence the production of these hypothetical substances, it also seems reasonable to believe that genes would preferentially affect four mating types at once. Yet the available data on the Tetrahymena genes lend no support to this notion. The gene $mt^{(I)-}$ blocks the appearance of only mating type I (and perhaps VIII, if this type is subsequently found); the gene $mt^{(IV,\ VII)-}$ blocks the appearance of only IV and VII (and perhaps VIII). It is of course possible that these genes are not concerned with the production of primary mating type substances but rather with other cellular conditions influencing which of the various substance-combinations will be expressed. Available information does not disprove METZ's hypothesis, but it offers it no support.

In the absence of information regarding the action of the mating type genes, their designation poses a problem. *A priori* the genes could be either "potentiating" or "inhibiting". Thus the $mt^{(I)-}$ factor could either inhibit the development of type I or it could make possible the production of types IV and VII (and perhaps others). The negative designations were chosen largely because they were simpler; they should not be interpreted as indicating anything regarding the mode of action of the genes. In fact, it would seem odd that allelic genes controlling inhibitors are mutually recessive. Of only one thing can we be reasonably sure; since mating type differences within a genotype are determined by persistent macronuclear differences, these genes must influence in some way the course of macronuclear differentiation.

Perhaps the chief significance of this study at the present time is the demonstration for *T. pyriformis* of a classical genetic mechanism, operating as would be expected from the cytological observations. Therefore, an essential step has been accomplished in preparing this organism for fruitful studies in genetics and cellular differentiation.

SUMMARY

Evidence is presented for the first nuclear genes to be found in *Tetrahymena pyriformis*. These genes, designated as $mt^{(I)-}$ and $mt^{(IV,\ VII)-}$, behave as alleles and act in determining mating type potentialities. Cells homozygous for the allele $mt^{(I)-}$ lack the ability to produce mating type I but can produce the other six mating types now known for this variety. Cells homozygous for the other allele lack the ability to produce types IV and VII. Any of the seven known mating types may be produced by heterozygous cells.

LITERATURE CITED

BUTZEL, H. M., JR., 1953 The genic basis of mating type determination and development in the varieties of *Paramecium aurelia* belonging to Group A. Thesis, Indiana University.

ELLIOTT, A. M., and D. F. GRUCHY, 1952 The occurrence of mating types in Tetrahymena. Biol. Bull. **103:** 301.

ELLIOTT, A. M., and R. E. HAYES, 1953 Mating types in Tetrahymena. Biol. Bull. **105:** 269–284.

GRUCHY, D. F., 1954 The system of breeding relations in *Tetrahymena pyriformis*. J. Protozoology **1** (suppl.): 2.

KIMBALL, R. F., 1942 The nature and inheritance of mating types in *Euplotes patella*. Genetics **27:** 269–285.

METZ, C. B., 1954 The physiology of fertilization in the Ciliates. A.A.A.S. Symposium, *Sex in Microorganisms*, ed. by D. H. WENRICH; pp. 284–334.

NANNEY, D. L., 1953 Nucleo-cytoplasmic interaction during conjugation in Tetrahymena. Biol. Bull. **105:** 133–148.

1954 Mating type determination in *Paramecium aurelia*, a study in cellular heredity. A.A.A.S. Symposium, *Sex in Microorganisms*, ed. by D. H. WENRICH; pp. 266–283.

NANNEY, D. L., and P. A. CAUGHEY, 1953 Mating type determination in *Tetrahymena pyriformis*. Proc. Nat. Acad. Sci. U. S. **39:** 1057–1063.

1955 An unstable nuclear condition in *Tetrahymena pyriformis*. Genetics **40:** 388–398.

RAY, C., JR., 1954 Chromosome behavior during conjugation of mating types I and II of variety 1 of Tetrahymena. Biol. Bull. **107:** 318–319.

SONNEBORN, T. M., 1937 Sex, sex inheritance and sex determination in *Paramecium aurelia*. Proc. Nat. Acad. Sci. U. S. **23:** 378–385.

1939 *Paramecium aurelia:* mating types and groups: lethal interactions: determination and inheritance. Am. Naturalist **73:** 390–413.

1947 Recent advances in the genetics of Paramecium and Euplotes. Advances in Genet. **1:** 263–358.

THE CORRELATIONS BETWEEN RELATIVES IN INBRED POPULATIONS[1]*

OSCAR KEMPTHORNE

Statistical Laboratory, Iowa State College, Ames, Iowa

Received February 4, 1955

A GENERAL approach to the problem of correlations between relatives under inbreeding is difficult even for the case of one locus. We can consider two distinct problems.

The first problem is that we have actually a random mating population

$$p^2\,AA + 2pq\,Aa + q^2\,aa$$

or the general one involving an arbitrary number of alleles. This population is inbred regularly, i.e. according to some regular system of inbreeding and at generation n we have the population

$$(p^2 + F_n pq)\,AA + 2(1 - F_n)pq\,Aa + (q^2 + F_n pq)\,aa$$

where F_n is the coefficient of inbreeding attained at generation n. We may ask the questions: what is the correlation between full sibs in generation n or, what is the correlation between an individual of generation n and his offspring in generation $(n + 1)$? In general the total genotypic variance changes in a complex manner through the generations. In fact the genotypic variance in generation n is equal to

$$pq\left(\frac{1 + F_n}{2}\right)y^2 - (1 - F_n)^2 p^2 q^2 x^2 + \tfrac{1}{2}(1 - F_n)pqx^2 + (1 - F_n)pq(p - q)xy$$

when the genotypic values of AA, Aa, and aa are coded as y, $(y - x)/2$ and 0 respectively.

The present paper deals with this problem with inbreeding by full-sibbing. Some aspects of this problem have been considered by ROBERTSON (1952).

A second problem is to suppose that the population

$$(p^2 + Fpq)\,AA + 2(1 - F)pq\,Aa + (q^2 + Fpq)\,aa$$

is in equilibrium under a regular system of inbreeding and our job is to find the correlation between relatives in this constant population. The difficulty with this problem is that one guesses that there are a large number of regular systems of inbreeding which would result in the population being at equilibrium. It is curious that this problem has not been worked on extensively. WRIGHT (1921) showed that with mating of half first cousins F tends to 1/27, and with mating of second cousins F tends to 1/53. A few remarks are all that we have to offer. Consider the situation given in figure 1. We suppose that $F_x = F_y = F_z$, so that the population is stationary with respect to the inbreeding coefficient.

[1] Journal Paper No. J-2700 of the Iowa Agricultural Experiment Station, Ames, Iowa Project 890.

* Part of the cost of the mathematical formulae has been paid by the GALTON AND MENDEL MEMORIAL FUND.

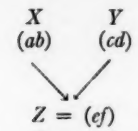


FIGURE 1

The array for Z is

$$(ef) = \tfrac{1}{4}(ac) + \tfrac{1}{4}(ad) + \tfrac{1}{4}(bc) + \tfrac{1}{4}(bd)$$

We now proceed to consider the covariance of X and Z. We wish to calculate $E(y_{ab}y_{ef})$ and to do this we need to know the probabilities of all the possible likenesses by descent of a, b, e, f or of a, b, c, d. For instance we need to know $P(a = b = c = d)$, $P(a = b = c \neq d)$ and so on, where $a = b$ denotes that genes a and b are identical by descent. These probabilities are not all specified merely by the fact that the population is in equilibrium at a constant level of inbreeding. The solution of the second problem does not appear therefore to be easy and has not been given in the literature.

We now revert to the first problem, that is the problem of the covariance between relatives after n generations of a regular system of inbreeding, in particular, full-sibbing.

COVARIANCES AMONG RELATIVES UNDER FULL SIBBING

We shall give the solution only for the case of two alleles A, a and we shall suppose that we have initially the population

$$p^2\,AA + 2pq\,Aa + q^2\,aa$$

and that after matings are made at random initially all subsequent matings are between full sibs. We shall derive the covariance between full sibs after n generations.

It is necessary to use the methodology of the theory of inbreeding given by FISHER (1949), some of which is due to earlier writers, and the reader should refresh his mind on that material before continuing. In the case dealt with by FISHER, concern was only with mating types, where the matings $AA \times AA$ and $aa \times aa$ for instance are of the same type. We now need to consider all the possible matings with respect to the genotypes of the individuals in the matings. We therefore talk about kinds of matings. The use of the phrase "kind of mating" here is purely a convenience, and some other phrase may be better. The kinds of matings with initial frequencies are as follows:

Mating		*Initial Frequency*
$AA \times AA$	(1)	p^4
$AA \times Aa$	(2)	$4p^3q$
$AA \times aa$	(3)	$2p^2q^2$
$Aa \times Aa$	(4)	$4p^2q^2$
$Aa \times aa$	(5)	$4pq^3$
$aa \times aa$	(6)	q^4

We let the frequencies of the kinds of matings in generation n be $f_1^{(n)}, f_2^{(n)}, \cdots f_6^{(n)}$. Then we have the equations

$$\begin{aligned}
f_1^{(1)} &= f_1^{(0)} + \tfrac{1}{4} f_2^{(0)} + \tfrac{1}{16} f_4^{(0)} \\
f_2^{(1)} &= \tfrac{1}{2} f_2^{(0)} + \tfrac{1}{4} f_4^{(0)} \\
f_3^{(1)} &= \tfrac{1}{8} f_4^{(0)} \\
f_4^{(1)} &= \tfrac{1}{4} f_2^{(0)} + f_3^{(0)} + \tfrac{1}{4} f_4^{(0)} + \tfrac{1}{4} f_5^{(0)} \\
f_5^{(1)} &= \tfrac{1}{4} f_4^{(0)} + \tfrac{1}{2} f_5^{(0)} \\
f_6^{(1)} &= \tfrac{1}{16} f_4^{(0)} + \tfrac{1}{4} f_5^{(0)} + f_6^{(0)}
\end{aligned}$$

or in matrix form $f^{(1)} = A f^{(0)}$, where $f^{(1)}$ and $f^{(0)}$ are column matrices and A is a 6×6 matrix. The roots of the matrix are denoted in the following by

$$\lambda_1 = 1, \quad \lambda_2 = \tfrac{1}{2}, \quad \lambda_3 = \tfrac{1}{4}, \quad \lambda_4 = \epsilon = \tfrac{1}{4}(1 + \sqrt{5}), \quad \lambda_5 = \epsilon' = \tfrac{1}{4}(1 - \sqrt{5}), \quad \lambda_6 = 1$$

and principal components corresponding to these roots (denoted by FISHER as P, Q, R, L, M, Z) are

$$\begin{aligned}
g_1 &= f_1 + f_2 + f_3 + f_4 + f_5 + f_6 , \\
g_2 &= f_2 - f_5 , \\
g_3 &= f_2 - 4f_3 - f_4 + f_5 , \\
g_4 &= f_2 + (4 + 8\epsilon')f_3 - 4\epsilon' f_4 + f_5 , \\
g_5 &= f_2 + (4 + 8\epsilon)f_3 - 4\epsilon f_4 + f_5 , \\
g_6 &= 4f_1 + 3f_2 + 2f_3 + 2f_4 + f_5 ,
\end{aligned}$$

and the reader may verify directly that the following relationships hold:

$$g_1^{(n)} = g_1^{(0)}, \quad g_2^{(n)} = (\tfrac{1}{2})^n g_2^{(0)}, \quad g_3^{(n)} = (\tfrac{1}{4})^n g_3^{(0)}, \quad g_4^{(n)} = \epsilon^n g_4^{(0)}, \quad g_5^{(n)} = (\epsilon')^n g_5^{(0)}, \quad g_6^{(n)} = g_6^{(0)}.$$

Also, by solving for the f_i's in terms of the g_i's

$$\begin{bmatrix} f_1 \\ f_2 \\ f_3 \\ f_4 \\ f_5 \\ f_6 \end{bmatrix} = \begin{bmatrix} 0 & -\frac{1}{4} & -\frac{1}{20} & -\left(\frac{1}{8} + \frac{2}{5}\epsilon\right) & -\left(\frac{1}{8} + \frac{2}{5}\epsilon'\right) & \frac{1}{4} \\ 0 & \frac{1}{2} & \frac{1}{5} & \frac{1}{10}\left(1 + 2\epsilon\right) & \frac{1}{10}\left(1 + 2\epsilon'\right) & 0 \\ 0 & 0 & -\frac{1}{10} & \frac{1}{20} & \frac{1}{20} & 0 \\ 0 & 0 & -\frac{1}{5} & \frac{2}{5}\epsilon & \frac{2}{5}\epsilon' & 0 \\ 0 & -\frac{1}{2} & \frac{1}{5} & \frac{1}{10}\left(1 + 2\epsilon\right) & \frac{1}{10}\left(1 + 2\epsilon'\right) & 0 \\ 1 & \frac{1}{4} & -\frac{1}{20} & -\left(\frac{1}{8} + \frac{2}{5}\epsilon\right) & -\left(\frac{1}{8} + \frac{2}{5}\epsilon'\right) & -\frac{1}{4} \end{bmatrix} \begin{bmatrix} g_1 \\ g_2 \\ g_3 \\ g_4 \\ g_5 \\ g_6 \end{bmatrix}$$

where we have used the matrix form of representing the results. In this equation

$$f_j = \sum_k C^{jk} g_k$$

where C^{jk} is the number in the j^{th} row and k^{th} column of the 6×6 array on the right-hand side. Now the frequencies of kinds of mating are the frequencies of full sib pairs, when the mating system is full-sibbing. Hence we merely have to find the g's in the initial generation and hence the g's in the n^{th} generation and then find the f's. In fact we find

$$\begin{aligned}
g_1^{(0)} &= 1 \\
g_2^{(0)} &= 4pq(p - q) \\
g_3^{(0)} &= 4pq(p^2 - 3pq + p^2) \\
g_4^{(0)} &= 4pq \\
g_5^{(0)} &= 4pq \\
g_6^{(0)} &= 4p
\end{aligned}$$

so that

$$\begin{aligned}
g_1^{(n)} &= 1 \\
g_2^{(n)} &= (\tfrac{1}{2})^n 4pq(p^2 - q^2) \\
g_3^{(n)} &= (\tfrac{1}{4})^n 4pq(p^2 - 3pq + q^2) \\
g_4^{(n)} &= \epsilon^n\, 4pq \\
g_5^{(n)} &= \epsilon'^n\, 4pq \\
g_6^{(n)} &= 4p
\end{aligned}$$

The population genotypic array in any generation is equal to

$$(f_1 + \tfrac{1}{2}f_2 + \tfrac{1}{2}f_3)\, AA + (\tfrac{1}{2}f_2 + f_4 + \tfrac{1}{2}f_5)\, Aa + (\tfrac{1}{2}f_3 + \tfrac{1}{2}f_5 + f_6)\, aa.$$

Now

$$\begin{aligned}
f_1 + \frac{1}{2}f_2 + \frac{1}{2}f_3 &= \left[-\frac{1}{4}g_2 - \frac{1}{20}g_3 - \left(\frac{1}{8} + \frac{2\epsilon}{5}\right)g_4 - \left(\frac{1}{8} + \frac{2\epsilon'}{5}\right)g_5 + \frac{1}{4}g_6 \right] \\
&\quad + \left[\frac{1}{4}g_2 + \frac{1}{10}g_3 + \frac{1}{20}(1 + 2\epsilon)g_4 + \frac{1}{20}(1 + 2\epsilon')g_5 \right] \\
&\quad + \left[-\frac{1}{20}g_3 + \frac{1}{40}g_4 + \frac{1}{40}g_5 \right] \\
&= \left(-\frac{1}{20} - \frac{3\epsilon}{10} \right) g_4 + \left(-\frac{1}{20} - \frac{3\epsilon'}{10} \right) g_5 + \frac{1}{4}g_6
\end{aligned}$$

Hence in generation n

$$f_1^{(n)} + \frac{1}{2}f_2^{(n)} + \frac{1}{2}f_3^{(n)} = \left(-\frac{1}{20} - \frac{3\epsilon}{10}\right)g_4^{(n)} + \left(-\frac{1}{20} - \frac{3\epsilon'}{10}\right)g_5^{(n)} + \frac{1}{4}g_6^{(n)}$$

$$= \left(-\frac{1}{20} - \frac{3\epsilon}{10}\right)\lambda_4^n g_4^{(0)} + \left(-\frac{1}{20} - \frac{3\epsilon'}{10}\right)\lambda_5^n g_5^{(0)} + \frac{1}{4}\lambda_6^n g_6^{(0)}$$

$$= \left(-\frac{1}{20} - \frac{3\epsilon}{10}\right)\epsilon^n 4pq + \left(-\frac{1}{20} - \frac{3\epsilon'}{10}\right)\epsilon'^n 4pq + \frac{1}{4}\cdot 1^n 4p$$

$$= p - pq\left[\frac{1}{5}\epsilon^n + \frac{1}{5}\epsilon'^n + \frac{6}{5}\epsilon'^{n+1} + \frac{6}{5}\epsilon'^{n+1}\right]$$

or if we let

$$S_n = \epsilon^n + \epsilon'^n, \qquad S_{n+1} = \epsilon^{n+1} + \epsilon'^{n+1}$$

the frequency of AA individuals in generation n is

$$p - pq(\tfrac{1}{5}S_n + \tfrac{6}{5}S_{n+1}).$$

Similarly the frequency of Aa in generation n is

$$2pq(\tfrac{1}{5}S_n + \tfrac{6}{5}S_{n+1}),$$

and the frequency of aa is

$$q - pq(\tfrac{1}{5}S_n + \tfrac{6}{5}S_{n+1}).$$

If now we let

$$\tfrac{1}{5}S_n + \tfrac{6}{5}S_{n+1} = 1 - F_n$$

the genotypic array in generation n is

$$[p - (1 - F_n)pq]\,AA + 2(1 - F_n)pq\,Aa + [q - (1 - F_n)pq]\,aa$$

which is the standard result obtained by WRIGHT. We should note that the generation matrix method is not necessary to obtain this result. The result can be (and was) obtained by a rather elementary argument by WRIGHT (1921) and MALÉCOT (1948).

The mean in generation n using genotypic values of d, h, r for the genotypes AA, Aa and aa respectively is

$$\mu_n = pd + qr - (1 - F_n)\,pq(d - 2h + r).$$

Since the quantity $(d - 2h + r)$ which is in some sense a dominance deviation (actually a linear function of dominance deviations) will appear frequently in the ensuing development, we shall denote it by x for brevity. Also we denote $(d - r)$ by y. (This amounts to a recoding of genotypic values.) Hence

$$\mu_n = (pd + qr) - (1 - F_n)\,pqx.$$

The covariance between full sibs in generation n is equal by definition to

$$f_1^{(n)}\,d^2 + f_2^{(n)}\,dh + f_3^{(n)}\,dr + f_4^{(n)}\,h^2 + f_5^{(n)}\,hr + f_6^{(n)}\,r^2 - \mu_n^2.$$

We now put everything in terms of F's by the use of the following equation

$$S_{n+2} = \tfrac{1}{2}S_{n+1} + \tfrac{1}{4}S_n$$

which holds since ϵ and ϵ' are the roots of the equation

$$x^2 = \tfrac{1}{2}x + \tfrac{1}{4}.$$

Also by definition

$$\begin{aligned}(1 - F_n) &= \tfrac{1}{5}S_n + \tfrac{6}{5}S_{n+1} \\ &= \tfrac{1}{5}S_n + \tfrac{6}{5}(\tfrac{1}{2}S_n + \tfrac{1}{4}S_{n-1}) \\ &= \tfrac{3}{10}S_{n-1} + \tfrac{4}{5}S_n\end{aligned}$$

and

$$(1 - F_{n-1}) = \tfrac{1}{5}S_{n-1} + \tfrac{6}{5}S_n\,.$$

Hence

$$S_{n-1} = 2 + 4F_{n-1} - 6F_n$$

and

$$S_n = \tfrac{1}{2} - \tfrac{3}{2}F_{n-1} + F_n$$

The final result for the covariance between full sibs is

$$\begin{aligned}Cov(F\cdot S) = {} & \tfrac{1}{4}(1 + F_{n-1} + 2F_n)\ pq\ y^2 - (1 - F_n)^2 p^2 q^2 x^2 \\ & - (\tfrac{1}{2})^n\ pq(p - q)xy - \tfrac{1}{5}(\tfrac{1}{4})^n pq(p^2 - 3pq + q^2)x^2 \\ & + (\tfrac{3}{10} + \tfrac{1}{10}\ F_{n-1} - \tfrac{2}{5}\ F_n)\ pqx^2 + (1 - F_n)pq(p - q)xy\end{aligned}$$

Also the variance in generation n is

$$\begin{aligned}V(S) &= pd^2 + pr^2 - (1 - F_n)\ pq(d^2 - 2h^2 + r^2) - \mu_n^2 \\ &= pq(d - r)^2 - (1 - F_n)\ pq(d^2 - 2h^2 + r^2) - (1 - F_n)^2 p^2 q^2 x^2 \\ &\quad + 2(1 - F_n)pq(pd + qr)x \\ &= \frac{pq}{2}(1 + F_n)y^2 - (1 - F_n)^2 p^2 q^2 x^2 + \tfrac{1}{2}(1 - F_n)pqx^2 + (1 - F_n)pq(p - q)xy.\end{aligned}$$

The correlation between full sibs is then

$$Cov\ (F\cdot S)/V(S)$$

DISCUSSION OF RESULT

Relation to previous work

The problem herein considered was worked out by WRIGHT (1921) for the special case when there is no dominance. In that case we may put $d = 2a$, $h = a$, and $r = 0$, so that $x = d + r - 2h = 0$. This fact was the reason for the particular form in

which the result is given above. A number of unpleasant terms drop out. In fact we have

$$Cov\,(F\cdot S) = (1 + F_{n-1} + 2F_n)\,pqa^2$$

and

$$V(S) = 2(1 + F_n)\,pqa^2$$

and the correlation is

$$\frac{1}{2}\left[\frac{1 + F_{n-1} + 2F_n}{1 + F_n}\right] = \frac{1}{2}\left[1 + \frac{F_{n-1} + F_n}{1 + F_n}\right].$$

It may be noted also that the additive genetic variance in the original population is $2pqa^2$.

Special case $p = q = 1/2$

In this case the term involving the root 1/2 drops out. Also we shall make use of the MATHER terminology for genotypic values, with $d = d, h = h, r = -d$. Then $d - r = 2d$, $x = d + r - 2h = -2h$. We then find

$$\begin{aligned} Cov(F\cdot S) &= \frac{1}{4}(1 + F_{n-1} + 2F_n)\,d^2 - (1 - F_n)^2\frac{h^2}{4} \\ &\quad + \left(\frac{3}{10} + \frac{1}{10}F_{n-1} - \frac{2}{5}F_n\right)h^2 + \frac{1}{20}\left(\frac{1}{4}\right)^n h^2 \\ V(S) &= d^2 - \frac{1}{2}(1 - F_n)(d^2 - h^2) - (1 - F_n)^2\frac{h^2}{4} \\ &= \left(\frac{1 + F_n}{2}\right)d^2 + \frac{1}{4}(1 - F_n^2)h^2. \end{aligned}$$

These formulae may be used for the case when two inbred lines are crossed in a way analogous to the use suggested by MATHER (1949, pp. 55–58) for certain formulae he gives based on selfing. He denotes d^2 by D and h^2 by H.

General comment

The present problem is interesting in that it is one which has been tackled by the method of path coefficients under the very restrictive assumption that the genes are additive in their effects. The method of path coefficients in this as in all attacks on the theory of inbreeding leads to a subset of the roots of the generation matrix. The full solution of the present problem requires knowledge of all the latent roots of the generation matrix and is therefore another problem in the theory of inbreeding which requires this knowledge. It appears that, as is apparently true in general, the method of path coefficients cannot be adapted to the treatment of non-additive gene effects.

One may take the point of view that the only important part of the covariance of the full-sibs is that part which ignores the roots of the generation matrix apart from ϵ and ϵ' which are contained in the quantity F_n, the coefficient of inbreeding in gener-

ation n. After the full answer has been obtained one is then in a position to evaluate the contributions of the other roots to the covariance. If one could assume that only the dominant latent root merits taking into account the whole problem is much simpler. A discussion of these matters is given by KEMPTHORNE (unpublished).

On a quite different aspect of the matter, it should be noted that the variances and covariances do not depend on the additive genetic variance or the dominance variance in the original random mating population. Also it does not appear that they will depend only on the additive genetic variance and dominance variance of the population in any inbred generation. Actually in the present case, the additive genetic variance in the original population is

$$2\,pq[p(d-h)+q(h-r)]^2 = \tfrac{1}{2}pq\{y+(p-q)x\}^2$$

and the dominance variance is

$$p^2q^2x^2.$$

For the inbred population in generation n, the additive genetic variance is equal to

$$2\,pq(1+F)\alpha^2$$

where

$$\alpha = \frac{1}{(1+F)}\,[(d-r)-(1-F)\,\{q(d-h)-p(h-r)\}],$$

or in another form

$$pq\left(\frac{1+F}{2}\right)y^2 + (1-F)pq(p-q)xy + \frac{(1-F)^2}{2(1+F)}\,pq(p-q)^2x^2.$$

The dominance variance is the total genotypic variance minus the additive genetic variance, and is equal to

$$\left(\frac{1-F}{1+F}\right)pqx^2\,[F+(1-F)^2pq].$$

It appears therefore that the utility of the concepts of additive genetic variance and dominance variance is rather severely limited to the case of random mating populations. The uses of the concept of additive genetic values is discussed by FISHER (1941) and some extensions are given by KEMPTHORNE (unpublished).

The extension of the results given herein to the case of more than two alleles at a locus is a mathematical detail which will be sketched only in the present paper. Presumably the additional latent root equal to $-\frac{1}{8}$ found by FISHER (1949) will enter into the result, but this may not happen.

The extension to the case of an arbitrary number of unlinked loci when there is no epistacy is direct. One merely adds the variances and covariances over the whole set of loci. Strictly speaking the symbols D and H of MATHER (1949) stand for the sums over a set of loci of the quantities d^2 and h^2.

Parent-offspring covariances under full-sibbing

Most of the work already done is useful in obtaining the covariance of parents in generation n with offspring in generation $n+1$. In fact this is equal to

$$f_1^{(n)} d^2 + f_2^{(n)}[\tfrac{1}{2}(d+h)]^2 + f_3^{(n)} \tfrac{1}{2}(d+r)h + f_4^{(n)} h(\tfrac{1}{4}d + \tfrac{1}{2}h + \tfrac{1}{4}r) + f_5^{(n)}[\tfrac{1}{2}(h+r)]^2 + f_6^{(n)} r^2 - \mu_n \mu_{n+1}.$$

After some manipulation this reduces to

$$Cov(P\cdot O) = \frac{pq}{4}(1 + F_n + 2F_{n+1})(y^2) - (1 - F_n)(1 - F_{n+1})p^2q^2x^2 - \left(\frac{1}{2}\right)^{n+1} pq(p-q)xy + \frac{pq}{4}(1 - F_n)x^2 + \left(\frac{2 - F_n - F_{n+1}}{2}\right) pq(p-q)(xy).$$

Also we have

$$V(P) = pq\left(\frac{1 + F_n}{2}\right) y^2 + \tfrac{1}{2}(1 - F_n)pqx^2 - (1 - F_n)^2 p^2q^2x^2 + (1 - F_n)pq(p-q)xy,$$

and $V(O)$ is the same formula with F_{n+1} in place of F_n.

DISCUSSION OF RESULTS

Relation to previous work

With no dominance we may put $d = 2a$, $h = a$, $r = 0$, $x = 0$ and we find

$$Cov(PO) = pqa^2(1 + F_n + 2F_{n+1})$$
$$V(P) = 2pqa^2(1 + F_n)$$
$$V(O) = 2pqa^2(1 + F_{n+1}).$$

Hence

$$\rho_{PO} = \frac{1 + F_n + 2F_{n+1}}{2\sqrt{(1 + F_n)(1 + F_{n+1})}}, \qquad \beta_{OP} = \frac{1 + F_n + 2F_{n+1}}{2(1 + F_n)} = \frac{1}{2} + \frac{F_{n+1}}{(1 + F_n)}$$

These results are in accord with those of WRIGHT.

Special case $p = q = 1/2$

We then have the following results after putting $d = d$, $h = h$, $r = -d$:

$$Cov(P\cdot O) = \tfrac{1}{4}(1 + F_n + 2F_{n+1})d^2 + \frac{F_{n+1}}{4}(1 - F_n)h^2.$$

This result could have applicability to populations resulting from the F_2 of two inbred lines.

Comment

Essentially the same general comments apply to this case as to the case of full-sib covariances. It is however of interest to note that the root $\lambda = \frac{1}{4}$ makes no contribution to the parent-offspring covariance while it does to the full-sib covariance.

The general case of an arbitrary number of alleles

The situation with an arbitrary number of alleles at the locus can be written down from FISHER's work (1949). Consider an initial mating $(ab) \times (cd)$ which is in general of type (vii): if genes a and b are identical in state the mating type will of course be type (vi) and so on. Then the frequencies of FISHER's type (i) to type (vii) are, from his pages 36, 37, as follows

Type	*Frequency in generation n*
$aa \times aa$	$l_n = 1 - \frac{1}{10}(5S_n + 16S_{n+1}) + (\frac{1}{2})^n - \frac{1}{5}(\frac{1}{4})^n$
$aa \times ab$	$u_n = \frac{1}{5}(2S_n + 4S_{n+1}) - 2(\frac{1}{2})^n + \frac{4}{5}(\frac{1}{4})^n$
$ab \times ab$	$v_n = \frac{4}{5}S_{n+1} - \frac{4}{5}(\frac{1}{2})^n + \frac{4}{15}(\frac{1}{4})^n + \frac{2}{15}(-\frac{1}{8})^n$
$aa \times bb$	$w_n = \frac{1}{10}S_n - \frac{1}{5}(\frac{1}{2})^n + \frac{2}{15}(\frac{1}{4})^n - \frac{2}{15}(-\frac{1}{8})^n$
$ab \times ac$	$x_n = \frac{8}{5}(\frac{1}{4})^n - \frac{4}{3}(\frac{1}{4})^n - \frac{4}{15}(-\frac{1}{8})^n$
$aa \times bc$	$y_n = \frac{2}{5}(\frac{1}{2})^n - \frac{2}{3}(\frac{1}{4})^n + \frac{4}{15}(-\frac{1}{8})^n$
$ab \times cd$	$z_n = (\frac{1}{4})^n$

In the initial random mating $(ab) \times (cd)$, a, b, c, d will be alleles taken at random from the population so that

$$P(a = A) = p(b = A) = p(c = A) = p(d = A) = p_1$$

etc. Also the frequencies of the kinds of matings can be written down. For instance the mating type $(aa) \times (ab)$ will contain the following matings in equal proportions: $aa \times ab$, $aa \times ac$, $aa \times ad$, $bb \times ba$, $bb \times bc$, $bb \times bd$, $cc \times ca$, $cc \times cb$, $cc \times cd$, $dd \times da$, $dd \times db$, $dd \times dc$. Hence the frequency of each of these matings in generation n is equal to $u_n/12$. A similar argument may be applied to the other mating types. Finally the probabilities of the genes a, b, c, d being either of $A_1, A_2, \cdots, A_m$ the possible alleles at the locus may be used to give the array of matings by genotype in the n^{th} generation. It turns out that these are as follows for an arbitrary generation where p_i is the gene frequency of A_i.

Kind of Mating	*Frequency*
$A_iA_i \times A_iA_i$	$p_i l + p_i^2 (u + v + w) + p_i^3 (x + y) + p_i^4 z$
$A_iA_i \times A_iA_j$	$p_i p_j u + 2p_i^2 p_j x + 2p_i^2 p_j y + 4p_i^3 p_j z$
$A_iA_j \times A_iA_j$	$2p_i p_j v + (p_i^2 p_j + p_i p_j^2) x + 4p_i^2 p_j^2 z$
$A_iA_i \times A_jA_j$	$2p_i p_j w + (p_i^2 p_j + p_i p_j^2) y + 2p_i^2 p_j^2 z$
$A_iA_j \times A_iA_k$	$2p_i p_j p_k x + 8p_i^2 p_j p_k z$
$A_iA_i \times A_jA_k$	$2p_i p_j p_k y + 4p_i^2 p_j p_k z$
$A_iA_jA_kA_l$	$8p_i p_j p_k p_l z$

In order to write out frequencies for generation n, we substitute t_n for t, u_n for u and so on from above. General formulas for covariances can be written down but they are useless in any form so far obtained.

SUMMARY

The problem of correlations between relatives under inbreeding is discussed. Solutions have been obtained for the genotypic covariances in the n^{th} generation of inbreeding from the random mating populations $p^2\,AA + 2pq\,Aa + q^2\,aa$. The covariance between full-sibs after n generations of full-sibbing and the covariance between parent in the n^{th} generation and offspring in the $(n+1)^{th}$ generation are given. It is seen that these depend on the latent roots of the generation matrix and not only on the roots $\epsilon = \frac{1}{4}(1+\sqrt{5})$ and $\epsilon' = \frac{1}{4}(1-\sqrt{5})$ which are sufficient to describe the change in the proportion of heterozygous individuals. The full-sib covariance depends on all the latent roots, at least in the situation examined, while the parent-offspring covariance depends on all the latent roots but one. It is also seen that the covariances do not depend only on the additive genetic variance and the dominance variance in the original random mating population, or in any derived population.

LITERATURE CITED

FISHER, R. A., 1941 Average excess and average effect of a gene substitution. Ann. Eug. **11:** 53–63.

1949 *The Theory of Inbreeding*. Oliver and Boyd, Edinburgh.

MALÉCOT, G., 1948 *Les mathématiques de l'hérédité*. Masson et cie, Paris.

MATHER, K., 1949 *Biometrical Genetics*. Dover, New York.

ROBERTSON, A., 1952 The effect of inbreeding on the variation due to recessive genes. Genetics **37:** 189–207.

WRIGHT, S., 1921 Systems of mating II. The effects of inbreeding on the genetic composition of a population. Genetics **6:** 124–143.

MODIFICATION OF X-RAY MUTAGENESIS IN DROSOPHILA. I. REUNION OF CHROMOSOMES IRRADIATED DURING SPERMIOGENESIS[1]

I. I. OSTER

Department of Zoology,[2] Indiana University, Bloomington, Indiana

Received February 7, 1955

MULLER (1938, 1939 a, b, 1940) studied the effect of variations in the timing of X-irradiation applied to mature Drosophila spermatozoa stored in the seminal receptacles and spermathecae of females and found no difference in the frequency of induced translocations whether the total dose of 2500r was delivered within a period of 25 minutes, divided into four fractions separated by intervals of a week, or extended throughout a month at an intensity of .01r per minute. His finding that such radical differences in timing did not alter the frequency of rearrangements led him to conclude that the broken ends of the spermatozoon's condensed chromosomes do not undergo union before the time of fertilization. Later, using similar techniques, DEMPSTER (1941) and KAUFMANN (1941) confirmed these findings. On the other hand, CATSCH and RADU (1943) presented evidence that union of broken ends does occur in not fully "mature" sperm. In their work irradiation of males with a dose of 4000r delivered within an interval of either 1 hour, 5 hours, or 10 hours and the subsequent testing for translocations in sperm released 4 to 6 days after being X-rayed resulted in the production of fewer translocations in the series in which the X-ray dose was applied over a protracted period of time. They interpreted this to indicate that some of the broken ends of chromosomes produced during the first few hours of the experiment had undergone union before having had a chance to unite with the ends from breaks which were produced during the last few hours of treatment.

Recent experiments by LÜNING (1952 a, b, c, d) and the similar but more definitive ones by AUERBACH (1954) have shown that the less mature stages of spermiogenesis are less resistant to radiation-induced damage than the more mature sperm and that even younger sperm are more resistant or at least exhibit fewer detectable changes. AUERBACH also found that the method and frequency of breeding which was used to obtain samples of sperm for analysis largely determined whether this period of increased sensitivity was detected at all. On the basis of these findings it is possible that the differences observed by CATSCH and RADU may have been due solely to variations in sperm utilization causing the sample analyzed for translocations to come from the more resistant sperm in the fractionated series and from more sensitive sperm in the other series. Because of this and because CATSCH and RADU failed to detect any increase in the frequency of translocations in sperm released a few days

[1] Adapted from a thesis presented for the partial fulfillment of the requirements for the degree of Doctor of Philosophy at Indiana University. This work was supported by a grant, for work of PROFESSOR H. J. MULLER and associates, from the Atomic Energy Commission (At (11-1)-195), and by a grant from the British Empire Cancer Campaign administered by C. AUERBACH.

[2] Contribution No. 581.

after irradiation in any of their work it was felt desirable to redetermine the time of union of breaks produced in immature spermatozoa by using a technique less liable to be complicated by the variations mentioned above.

It was thought that irradiation of spermatozoa stored in females should provide us with a more nearly homogeneous sample of mature sperm and that irradiation of pupae accurately classified according to age in which there is a preponderance of one or another stage of germ-cell development should provide us with a sample of the earlier stages of spermiogenesis. AUERBACH had shown the latter technique to be ideally suited for treating a given stage of gametogenesis and to be independent of the complications arising from the irradiation of mature males containing all the stages of spermato- and spermiogenesis and the resulting mixing of sperm which was at different stages of development at the time of treatment and less subject to variations in its utilization.

METHODS

Flies of a stock containing a ring-X chromosome (X^{c2}) in the male and yellow (*y*), forked (*f*) attached-X chromosomes in the female (*yf*:=) were allowed to oviposit for 1 hour on standard Drosophila medium. Five days later males which were in the so-called prepupal stage characterized by a fully formed but colorless puparium were collected and separated into two groups. As this stage of pupal development lasts less than 1 hour it serves as an accurate time mark from which to date the age of the pupae. One group of pupae was irradiated with an X-ray dose of 800r (135 kV and 1 mm thick aluminum filter) at 48 ± 2 hours after the onset of pupation (series I) together with a similarly collected group of pupae which was 72 ± 2 hours after puparium formation old (series II). Upon eclosion at 96 ± 2 hours after pupation, the treated (series I & series II) and untreated (series 0) imagoes were mated by placing 1 male with 3 *y In49 v*; *bw*; *e* virgin females in separate vials for 2 days (brood 1). After this period the males and females were separated and the inseminated females from series 0, I, and II were irradiated simultaneously with a dose of 1800r. These females were again allowed to lay eggs until their supply of sperm was exhausted (brood 2). The F_1 males were tested for translocations between autosomes II and III by being backcrossed to *y In49 v*; *bw*; *e* virgin females and examining the F_2 offspring for the absence of independent assortment between brown (*bw*) and ebony (*e*) which are not linked in normal flies and the F_1 females were tested for lethals by being mated to *y In49 v*; *bw*; *e* males and examining the F_2 offspring for the absence of non-yellow males which would indicate that a lethal had been induced in the paternal X chromosome. All the F_1 cultures which did not yield an adequate number of flies to enable us to determine whether or not a lethal or translocation was present were retested.

RESULTS

The genetic analysis of the three differently treated series of flies yielded the results shown in tables 1 and 2.

Our data are in agreement with the findings of LÜNING and AUERBACH in showing that irradiation with 800r of the stage of gametogenesis present in 48-hour old pupae

TABLE 1

Frequency of sex-linked lethals

Brood 1	Series 0 (Unirradiated)	Series I (Irradiated 48 ± 2 hour old pupae—800r)	Series II (Irradiated 72 ± 2 hour old pupae—800r)
Lethal X chromosomes	0	46	54
Total tested X chromosomes	500	798	2149
% lethals	0	5.76	2.51
Brood 2	**(Irradiated mature spermatozoa—1800r)**	**(Irradiated mature spermatozoa—1800r)**	**(Irradiated mature spermatozoa—1800r)**
Lethal X chromosomes	49	43	25
Total tested X chromosomes	703	284	325
% lethals	6.97	15.14	7.69

TABLE 2

Frequency of translocations involving chromosomes II and III

Brood 1	Series 0 (Unirradiated)	Series I (Irradiated 48 ± 2 hour old pupae—800r)	Series II (Irradiated 72 ± hour old pupae—800r)
Translocations of chromosomes II and III	0	44	17
Total tested sets of paternal chromosomes	0	763	1565
% Translocations	0	5.77	1.09
Brood 2	**(Irradiated mature spermatozoa—1800r)**	**(Irradiated mature spermatozoa—1800r)**	**(Irradiated mature spermatozoa—1800r)**
Translocations of chromosomes II and III	43	25	38
Total tested sets of paternal chromosomes	505	225	257
% Translocations	8.73	11.11	15.95

leads to the production of a higher frequency of lethals and translocations than the application of an equivalent dose of X-rays to older pupae does; these increased frequencies are nearly equal to those produced by treating more mature spermatozoa with a dose of 1800r. Histological examinations by KHISHIN (AUERBACH 1954 and unpublished) have demonstrated that the anterior portions of the testis of 48-hour old pupae contain mainly spermatid and spermatid transforming stages and that irradiation of such individuals results in the production of a high frequency of changes in their cells. Upon eclosion, these treated cells, being at the end of the testis closest to the genital opening are released first with little or no admixture of cells which were at an earlier stage of development (and hence less sensitive) at the time of irradiation.

When the immature spermatozoa of 48-hour pupae were irradiated and then these cells were given an additional dose of X-rays when they had become transformed into

mature spermatozoa, the frequencies of lethals and translocations were increased in only an additive manner. A non-synergistic effect for sex-linked lethal mutations after a fraction of the dose was applied to pupae and the other portion to the sperm in the male was reported previously by TIMOFÉEFF-RESSOVSKY (1934), TIMOFÉEFF-RESSOVSKY and ZIMMER (1935), and KERKIS (1938). However, judging from the relatively low mutation rates obtained after the pupal treatments, their experiments correspond to our series II in which the initial radiation was applied to nearly mature spermatozoa in 72-hour old pupae with similar results. While at the doses used an appreciable number of the lethal mutations are probably not due to 2-break deficiencies and thus an additive result for them is not too decisive, the fact that in our experiments even the translocations were additive indicates that the breaks produced by the first dose had already undergone union by the time that those produced by the second dose were able to reunite.

On the other hand, series II illustrates the inability of breaks induced by irradiation of the almost mature spermatozoa of the 72-hour pupae to undergo recombination. For in this case the translocation frequency, arising when this treatment was followed by irradiation of the same spermatozoa later in the female, was significantly greater than a merely additive result would have given. This shows that breaks produced by the 72-hour pupal irradiation were still "open" at the time when those produced by the irradiation of the sperm in the female were able to undergo union, for cross-union with the latter. Since the earlier evidence, previously cited, shows the latter breaks undergo union only after fertilization, it might be concluded that those induced in the 72-hour pupae likewise remain open until that stage.

In general our results tend to confirm the previous findings of MULLER who found that the condensed chromosomes of mature spermatozoa do not undergo union before fertilization and CATSCH and RADU who reported that the ends of chromosomes broken in less "mature" spermatozoa recombine prior to fertilization. However, as mentioned previously, the latter's results may have been fortuitous and are subject to an alternative explanation, i.e., that in reality they were comparing sensitive with less sensitive cells. Also, it is not possible to rule out as an interpretation of their work the one which LANE has presented to explain a similar reduction in the frequency of translocations. In his plant material he found a seemingly antagonistic effect of fractionated treatments and from this he reasoned that an earlier irradiation may increase the resistance of chromosomes to damage by a later irradiation. On the other hand, our experiments on the induction of lethal mutations and translocations in the same individuals, which yielded additive frequencies for the former and more than an additive one for translocations in the series involving irradiation of spermatids, indicate that an enhancement of radio-resistance was not produced.

Although our present experiments were not designed to throw light on the factors responsible for the increased sensitivity of spermatids to X-rays, it is possible that the drastic physical changes which occur during spermiogenesis are one of the main underlying causes. That is, during the elongation of the spherical spermatid to form the conical spermatozoon and the consequent moving of the chromosomes and probable separation of their parts if broken, reunion rather than restitution would be favored.

SUMMARY

The ends of chromosomes broken during spermiogenesis undergo reunion before fertilization while the breaks produced in mature spermatozoa remain open and reunite during fertilization. In these stages of germ cell development prior irradiation does not lead to an alteration in their reactions to subsequent doses of X-rays.

ACKNOWLEDGEMENTS

I wish to express my sincere appreciation to PROFESSOR H. J. MULLER for his constant encouragement and helpful suggestions during the course of these experiments. I would also like to thank DR. C. AUERBACH for many stimulating discussions along the lines of the present investigation.

LITERATURE CITED

AUERBACH, C., 1954 Sensitivity of the Drosophila testis to the mutagenic action of X-rays. Zeit. Ind. Abst. Ver. **86:** 113–125.

CATSCH, A., and GH. RADU, 1943 Die Abhängigkeit der röntgeninduzierter Translokationsrate bei *Drosophila melanogaster* von der Intensität der angewandten Bestrahlungsdosis. Naturwissenschaften **31:** 419–420.

DEMPSTER, E. R., 1941 Absence of a time factor in the production of translocations in Drosophila sperm by X-irradiation. Am. Naturalist **75:** 184–187.

KAUFMANN, B. P., 1941 The time interval between X-irradiation of sperm of Drosophila and chromosome recombination. Proc. Nat. Acad. Sci. U. S. **27:** 18–24.

KERKIS, J. J., 1938 (On the possibility of increasing the gene mutability by X-ray treatment of the organism at the early stage of development.) Izv. Akad. Nauk SSSR. [Otd. mat.-est., Ser. biol.]: 67–74.

LANE, G. R., 1951 X-ray fractionation and chromosome breakage. Heredity **5:** 1–35.

LÜNING, K. G., 1952a X-ray induced dominant lethals in different stages of spermatogenesis in Drosophila. Hereditas **38:** 91–107.

1952b X-ray induced chromosome breaks in *Drosophila melanogaster*. Hereditas **38:** 321–338.

1952c Studies on the origin of apparent gene mutations in *Drosophila melanogaster*. Acta Zool. **33:** 193–207.

1952d Studies on X-ray induced mutations in various stages of spermatogenesis in *Drosophila melanogaster*. Doctoral Dissertation, University of Stockholm, A. Bonnier's Press, Stockholm.

MULLER, H. J., 1938 The remaking of chromosomes. Collecting Net (Woods Hole) **13:** 181, 183–195, 198.

1939a Report of investigations with radium. Med. Res. Council Special Report Ser. **236:** 14–15.

1939b The mechanism of structural change in chromosomes of Drosophila. (Abstr.) Proc. 7th Internat. Genet. Congr., Edinburgh, (J. Genetics Suppl.), 221–222. (1941)

1940 An analysis of the process of structural change in chromosomes of Drosophila. J. Genet. **40:** 1–66.

TIMOFÉEFF-RESSOVSKY, N. W., 1934 Einige Versuche an *Drosophila melanogaster* über die Beziehungen zwischen Dosis und Art der Röntgenbestrahlung und der dadurch ausgelösten Mutationsrate. Strahlentherapie **49:** 463–478.

TIMOFÉEFF-RESSOVSKY, N. W., and K. G. ZIMMER, 1935 Strahlengenetische Zeitfactorversuche bei *Drosophila melanogaster*. Strahlentherapie **53:** 134–138.

A COMPARISON OF X-RAY AND ULTRAVIOLET EFFECTS ON CHROMOSOMES OF ZEA MAYS[1]

M. H. EMMERLING[2]

University of Missouri, Columbia, Missouri

Received March 3, 1955

PREVIOUS workers have found that in maize the genetic analyses have indicated an apparent difference between the action of X-ray and ultraviolet on (1) gene mutation, (2) the frequency of reciprocal translocations, (3) the type and frequency of endosperm deficiencies, (4) the ratio of endosperm deficiencies to embryo abortions, and (5) the types of embryo deficiencies. These contrasts have been fully discussed by STADLER and co-workers in a series of papers from 1936 to 1951 (STADLER and SPRAGUE 1937; STADLER 1939 and 1941; STADLER and ROMAN 1943 and 1948; STADLER and SWANSON 1951). This discussion will include only those points that have a direct bearing on the differences in the chromosomal aberrations induced by the two radiations.

In *Zea mays* the frequency of reciprocal translocations is low following treatment with ultraviolet radiation in contrast to the frequency found following X-ray treatment (STADLER and SPRAGUE 1937; STADLER 1941). STADLER and SPRAGUE found reciprocal translocation figures at diakinesis in 42% of the 100 plants from X-rayed pollen and no translocations in the same number of plants from ultraviolet treated series. In these experiments the doses of X-rays and ultraviolet radiation used were about equally effective in producing endosperm deficiencies.

Not only does it seem that the frequency of reciprocal translocations is different following treatment with ultraviolet radiation, but it has been suspected that the type of translocation is different (STADLER 1941). SINGLETON and CLARK (1940) recognized a type of translocation associated with a genic deficiency, called a deficiency translocation, in the progeny of ultraviolet treated pollen. A limited amount of evidence is available on the comparative frequency of deficiency translocations following X-rays and ultraviolet radiation. DEBOER (1945) found two deficiency translocations among 3513 plants following treatment with ultraviolet radiation but five deficiency translocations in 1670 plants from X-rayed pollen.

When the occurrence of interstitial and terminal chromosomal deficiencies following X-rays and ultraviolet radiation is compared, a difference is found. Following the use of ultraviolet no cytologically proven cases of interstitial deficiencies have been reported. However, several interstitial deficiencies have been observed in plants from X-rayed pollen (MCCLINTOCK 1931; DEBOER 1945). A number of terminal deficiencies have been reported in progeny following treatment with ultraviolet radiation (STAD-

[1] This work was supported by a grant from the Atomic Energy Commission. Contract AT (11-1)-73 project No. 4.

Submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Graduate School of the University of Missouri.

[2] Present address: Botany Department, University of Illinois, Urbana, Ill.

LER 1941; SINGLETON 1939; SINGLETON and CLARK 1940; and DEBOER 1945). Only in DEBOER's investigation is it certain that the chromosome loss was terminal because she was the only one to use a terminal marker. The latter is essential since an interstitial deficiency could appear as a terminal deficiency if non-homologous pairing occurred. Terminal deficiencies have been found in cytologically marked stocks from plants treated with X-rays (MCCLINTOCK 1931; and DOLLINGER 1954).

It seemed desirable, therefore, to make a more extensive study of the types of simple and complex chromosomal deficiencies which occur after X-ray and ultraviolet radiation and also to make a comparison of their frequency. It is important to emphasize that chromosomal rearrangements not involving a deficiency, such as reciprocal translocations and inversions, were not recovered in these experiments since the cases for cytological analysis were selected on the basis of a genetic deficiency.

MATERIALS AND METHODS

The cytological analysis was made on chromosomes 9 and 10. Stocks to be irradiated had the end of the short arm of chromosome 9 terminating in a knob (symbol *K*), either a large one, a medium sized one or a small one, all of these being easily distinguishable from a non-knobbed chromosome 9. The knob on the end of the short arm makes it possible to determine whether the irradiation causes terminal deficiencies. In order to select plants which had radiation damage in the vicinity of the knob, stocks homozygous for the dominant allele of the gene bronze (*bz*), which is located 31 units from *K*, were employed. Pollen from *Bz K/Bz K* plants, after appropriate irradiation, was placed on the silks of *bz k/bz k* (*k* denoting either a smaller knob than *K*, or no knob). The F_1 plants which exhibited the bronze phenotype were selected for study. Presumably this chromosome would be deficient for the dominant allele, *Bz*, as a result of the irradiation. In some families, it was possible to examine the seedlings for bronze variegation, but usually only a small fraction of the total population was examinable. These variegated seedlings were saved also for cytological examination.

For the study of the effects of irradiation on chromosome 10 a stock possessing the abnormal knob 10, as described by LONGLEY (1938), was used. Its subterminal position makes it impossible to establish with certainty whether the deficiency is terminal or interstitial. The stock homozygous for the knob was also homozygous for the dominant allele R^r (one unit from *K*) which produces anthocyanin in the plant. Pollen from such a stock $R^r\ K/R^r\ K$ was irradiated and placed on the silks of $r^g\ k/r^g\ k$ plants (green plants). The F_1 seedlings showing the phenotype of the egg parent were selected for cytological study.

The source of ultraviolet radiation used in these experiments was a Westinghouse Sterilamp WL782H-10 without a filter. Approximately 80% of the energy emitted by this lamp is in the region of 2537 Angstroms, which STADLER and UBER (1942) had found was the genetically most effective part of the ultraviolet spectrum. The dose used in this experiment was approximately 24,000 milliwatts per second per square foot of the treated surface obtained by placing the pollen in a single layer on a quartz slide. The pollen was irradiated from above and below for 10, 20, and 30 second exposures.

TABLE 1

The type and frequency of cytological aberrations in chromosome 9 following ultraviolet and X-ray treatment

Treatment	*n*	*bz*	Terminal df.	Interstitial df.	Fragment chr?	Ring chr.	Deficiency transloc.	Asynapsis	Haploid	Normal chr. 9	Died before meiosis	Unexam.
U.V. 10″	1143	2	1									1
20″	2948	7	4		1				2			
30″	2077	4	3							1		
Total	6168	13	8		1				2	1		1
X-ray 400r	1936	11		1	1		6					3
800r	1003	19	2	2	2	1	4	1		3	3	1
1000r	67	1									1	
Total	3006	31	2	3	3	1	10	1		3	4	4
Control	6397	1							1			

The X-radiation used was obtained from a Coolidge tube with a tungsten target producing radiation in the region of 0.605 Angstroms. The dosages were 400r, 800r, and 1000r.

The meiotic chromosomes were prepared for study by the aceto-carmine smear technique following fixation in a 3:1 mixture of ethyl alcohol (95%) and glacial acetic acid.

OBSERVATIONS

Effects of ultraviolet radiation on chromosome 9

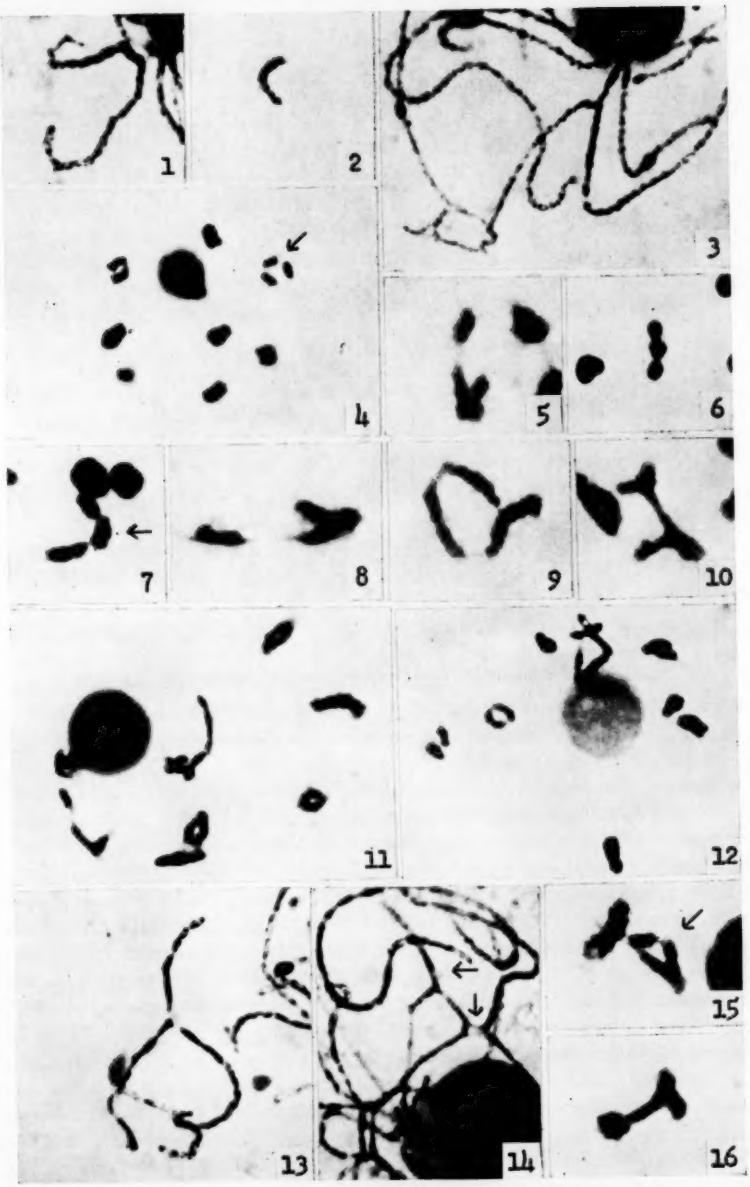
Three F_1 populations totalling 6168 plants were grown from crosses of *bz k/bz k* plants with *Bz K/Bz K* plants, the pollen of which had been irradiated with ultraviolet light for the three periods of time described previously. Thirteen seedlings were bronze indicating that there was either a loss or an inactivation of *Bz*. Twelve were examined cytologically (table 1).

Two of the 12 bronze F_1 plants were haploids, as was the one bronze plant observed in the control population.

Three of the 12 aberrant F_1 plants were found to have lost the whole of the short arm of chromosome 9. Five of the bronze plants had lost the terminal knob and part of the short arm of chromosome 9. One had lost about three quarters of the arm, one about two thirds, another about one half, another about one third, and the last, about one sixth. In all five cases the terminal knob was certainly missing, as can be seen in the one example shown in figure 1.

In one *bz* plant, the treated chromosome 9 was represented in pachytene by a small fragment (fig. 2). In ten pachytene figures, the centromere of this aberrant chromosome was observed in the same position, which would not be true if this were a ring chromosome. In a collapsed ring chromosome, the location of the centromere would vary according to the points at which the ring folded on itself.

One bronze plant from the ultraviolet treated progeny possessed a normal chromo-



FIGS. 1–16

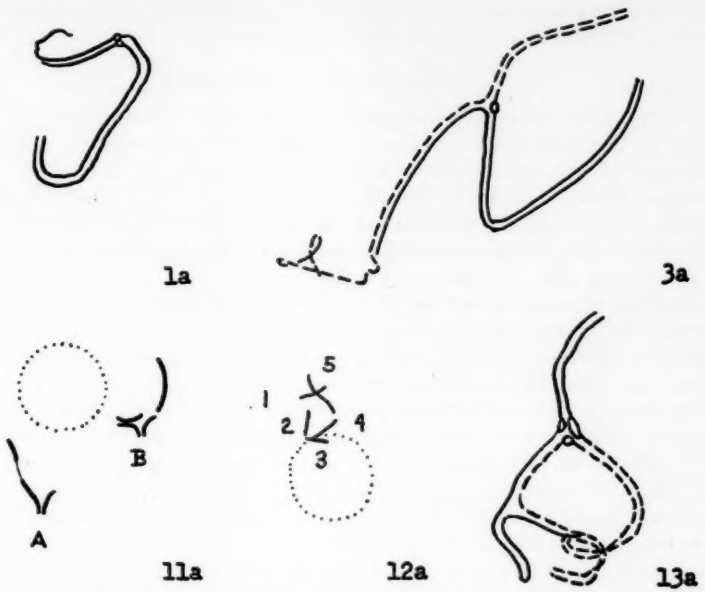


FIGURE 1a.—Diagram of figure 1. The centromeres are represented in the sketch as two circles. FIGURE 3a.—Diagrammatic interpretation of figure 3. The normal chromosome 9 is represented as a solid line; the normal unidentified chromosome is drawn as broken lines; the dicentric chromosome is illustrated by partly broken and solid lines. FIGURE 11a.—Diagram of figure 11. The 3-chromosome translocation (A) consists of three associated chromosomes and the reciprocal translocation (B) of four elements. FIGURE 12a.—Diagram of figure 12 showing the five elements of the translocation. FIGURE 13a.—Diagram of figure 13. The short arm of normal 10 (solid line) is paired homologously with the translocated chromosome (partly solid and broken lines), and the long arm of ten is paired non-homologously with the normal unidentified chromosome. The latter chromosome is also partially synapsed (broken lines) with the translocated chromosome.

FIGURES 1–3.—(× 1300) FIGURE 1.—Terminal deficiency for chromosome 9; see diagram. FIGURE 2.—Fragmented chromosome 9. FIGURE 3.—3-chromosome deficiency translocation involving chromosome 9 and an unidentified chromosome; see diagram. FIGURE 4.—Diakinesis cell with a 3-chromosome deficiency translocation indicated by an arrow (× 1600). FIGURES 5–10.—(× 2600) 3-chromosome deficiency translocation at diakinesis. FIGURE 11.—Diakinesis cell including a 3-chromosome deficiency translocation and a reciprocal translocation; see diagram (× 1600). FIGURE 12.—Type of translocation involving three chromosomes; see diagram (× 1600). FIGURE 13.—3-chromosome deficiency translocation involving chromosome 10 and an unidentified chromosome; see diagram (× 1300). FIGURE 14.—Same translocation in another cell. The two pairs of centromeres (indicated by arrows) are widely separated (× 1300). FIGURE 15.—3-chromosome deficiency translocation at diakinesis (× 2600). FIGURE 16.—3½-chromosome deficiency translocation (× 2600).

some 9 and a normal sized terminal knob. Unfortunately, this plant produced no progeny and thus the analysis could not be extended further.

Effects of X-ray radiation on chromosome 9

Mature pollen from plants homozygous for *Bz K/Bz K* was treated with X-rays (400r, 800r, and 1000r) and pollinated on homozygous recessive plants, *bz k/bz k*. Thirty-one bronze seedlings were found among 3006 seedlings, four of which died before meiosis. Four other plants could not be analyzed because the cytological specimens were poor. These cases were lost since the majority of deficiencies in *Zea mays* are not transmissible in succeeding generations. The results of the study of the 23 remaining plants are presented in table 1.

Two plants had terminal deficiencies of the short arm on chromosome 9 which were analogous to those found in the study of the bronze plants produced from pollen subjected to ultraviolet radiation. In both cases approximately one half of the short arm and the terminal knob were deficient.

Three plants had interstitial deficiencies in the short arm of chromosome 9, the terminal knob being present, unchanged in size. No plants with interstitial deficiencies in this chromosome were observed in the progeny from the ultraviolet irradiated pollen. One of the three interstitial deletions involved approximately one fifth of the short arm of chromosome 9. In the other cases, the deleted segment included about one fourth and two thirds of the short arm.

In three *bz* plants, a fragment of chromosome 9 remained following irradiation. Of course, the possibility of a small ring chromosome is not completely excluded although 25 pachytene figures of the rod-shaped chromosomes were seen in the first case, ten pachytene figures in the second case, and eight in the third case. The constant position of the centromere in these fragments is not a conclusive criterion to distinguish a rod from a ring chromosome since the fragments are so minute that a slight variation in the position of the centromere would not be detected.

One *bz* plant exhibited asynapsis in late pachytene and diakinesis. An examination of an earlier stage of pachytene, when the chromosomes should be paired in asynaptic plants, was not possible since the cytological sample was limited in quantity. This case, therefore, remains undetermined for the alteration associated with chromosome 9. The asynapsis could be due to a coincident loss of another chromosome.

In one *bz* plant a ring chromosome was present. The ring included approximately three fourths of the entire ninth chromosome.

Three bronze plants were found with no visible cytological deficiency. These three cases have not been tested for exclusion of contamination.

Ten plants with the bronze phenotype had a type of chromosomal alteration which was not found among the 12 bronze plants in the ultraviolet series. These plants have a deficiency associated with a translocation. The type of aberration is called a deficiency translocation. In one type, part of one chromosome possessing the centromere is attached to part of another chromosome which possesses its centromere, thus making a dicentric chromosome. The terminal, acentric parts of both chromosomes are lost. In diakinesis, the dicentric chromosome and the two normal chromosomes from the female (nonirradiated) parent make a chain of three chromosomes. For

this reason this type of deficiency translocation will be called a 3-chromosome deficiency translocation. The other type will be called a 3½-chromosome deficiency translocation. This term applies to those translocations in which one acentric fragment is translocated and the other acentric fragment failed to be interchanged, thus leaving one chromosome deficient. In diakinesis a chain of four rather than three chromosomes is associated. The chain of four chromosomes consists of two normal with one translocated chromosome in between, plus a chromosome deficient for some part which being acentric, has been lost. Most of the deficiency translocations involving the *Bz* locus were studied in diakinesis stage only, whereas this type of aberration was analyzed more fully in the pachytene stage in the experiments involving the R^r locus.

Nine 3-chromosome deficiency translocations were identified in the *bz* plants. The 3½ type of deficiency translocation was not found in the chromosome 9 series.

Four of the ten bronze plants with a 3-chromosome deficiency translocation were analyzed in the pachytene and diakinesis stages. In these plants, both breaks probably occurred near the centromere and the centric pieces reunited to form a dicentric chromosome. The acentric fragments, which failed to be translocated, were lost. The distance between the two centromeres of the dicentric chromosome is not always an indication of the original breakage points. This was demonstrated in those deficiency translocations which occurred in variegated plants from the R^r series. In these plants, the breakage point on chromosome 10 was distal to the R^r locus, which is located near the end of the long arm. At pachytene, only the short arm of chromosome 10 and its centromere were present; the long arm was lost, presumably by the process of repeated breakage and fusion. Figure 3 shows the 3-chromosome deficiency translocation at pachytene in one of the plants from the *Bz* series. Three arms are present, two of which are the homologously paired arms, and the third arm represents parts of the two normal chromosomes paired non-homologously. Only one centromere was positively identified in this figure, but two centromeres were seen in other figures. In the diakinesis stage, each of these four plants exhibited 8 bivalents and three associated chromosomes. Three of these four plants are illustrated at diakinesis in figures 4 to 6.

Five of the ten plants with the bronze phenotype had a 3-chromosome deficiency translocation which was analyzed in the diakinesis stage only. In each of these five plants, three associated chromosomes were found (figs. 7–11). In one plant, a coincident reciprocal translocation was present also. Figure 11 shows the three associated chromosomes of a 3-chromosome deficiency translocation and the four associated chromosomes of a reciprocal translocation.

In another bronze plant three chromosomes were involved in a type of translocation. This translocation could be interpreted as either one double 3-chromosome deficiency translocation, which is unlikely, or one deficiency translocation and also a reciprocal translocation. The first should have four associated chromosomes at diakinesis and second type should have five associated chromosomes in the translocation. Among 104 diakinesis cells examined in this plant, 90 cells consisted of either four or five associated chromosomes (fig. 12) and 14 had four associated chromosomes. The uncertainty of the number of chromosomes involved in the translocation origi-

TABLE 2

The type and frequency of cytological alterations in chromosome 10 with the aberrant knob following treatment with X-rays and ultraviolet radiation

Treatment	n	r^g plants	Varieg. plants	Term. df?	Interst. df.	Df. transl.	Df. inv?	Univalent + frag?	Monosomic 10	Ring chr.	Haploid	Normal chr. 10	Died before meiosis	Unexam.
U.V. 10″	3614	14	3+2?	4	1	1					4	4	2	3
20″	2526	12	[1]	3		1					3	1	2	2
30″	1283	10	[1]	1		1					3	4		1
Total	7423	36	3+2?	8[2]	1	3[2]					10	9	4	6[3]
X-ray 400r	4272	39	3[4]		1	9[5]	1	1	1	3	10	5	7[6]	4
Control	5310	8[7]		1								7		

[1] No variegation, but only ⅛ were suitable for examination of coleoptile.
[2] Including one variegated plant.
[3] Including three r^g plants, one variegated plant, and two questionable variegated plants.
[4] Approximately three fourths of seedlings were examinable for variegation.
[5] Including two variegated plants.
[6] Including one variegated plant.
[7] Plant losses included two possible unstable mutants and one possible intermediate mutant.

nates from the association of chromosome six with the nucleolus because the centric constriction of the nucleolar organizer chromosome cannot be distinguished with certainty from a terminal chiasma.

Effects of ultraviolet radiation on chromosome 10

In the chromosome 10 series the gene R^r provided the genetic marker and the aberrant knob 10 furnished the cytological marker. Pollen of the $R^r\,K/R^r\,K$ stock was treated with ultraviolet radiation and placed on silks of homozygous recessive plants, $r^g\,k/r^g\,k$. Thirty-six r^g individuals were found among 7423 seedlings, four of which died before maturity. Three plants gave poor cytological specimens and thus were not analyzed. In addition to the 36 r^g seedlings, three were variegated and there were two others which were possibly variegated. The cytological analysis was made on 29 of the 36 r^g seedlings and on two of the three variegated seedlings. The other variegated seedling and the two questionable variegated seedlings included two with sterile tassels and one with a poor cytological specimen. A summary of the types of cytological aberrations observed in the r^g and variegated seedlings is presented in table 2 along with the results from plants irradiated with X-ray radiation and those from control pollinations.

Seven of the 29 r^g plants and one of the two variegated plants exhibited deficiencies for the long arm of chromosome 10 and the knob as did one r^g plant found in the control population. As mentioned previously, the knobbed-10 chromosome may not be used to identify true terminal deficiencies since a segment of euchromatin is located distal to the knob. One plant was deficient for approximately seven eighths of the long arm of chromosome 10, one lacked about two thirds of the long arm, two were deficient for approximately one half of the long arm, and three lacked about one third

of the long arm. The terminal deficiency found in the variegated plant was deficient for three fourths of the long arm. At diakinesis, this plant showed no translocation figure in 25 cells.

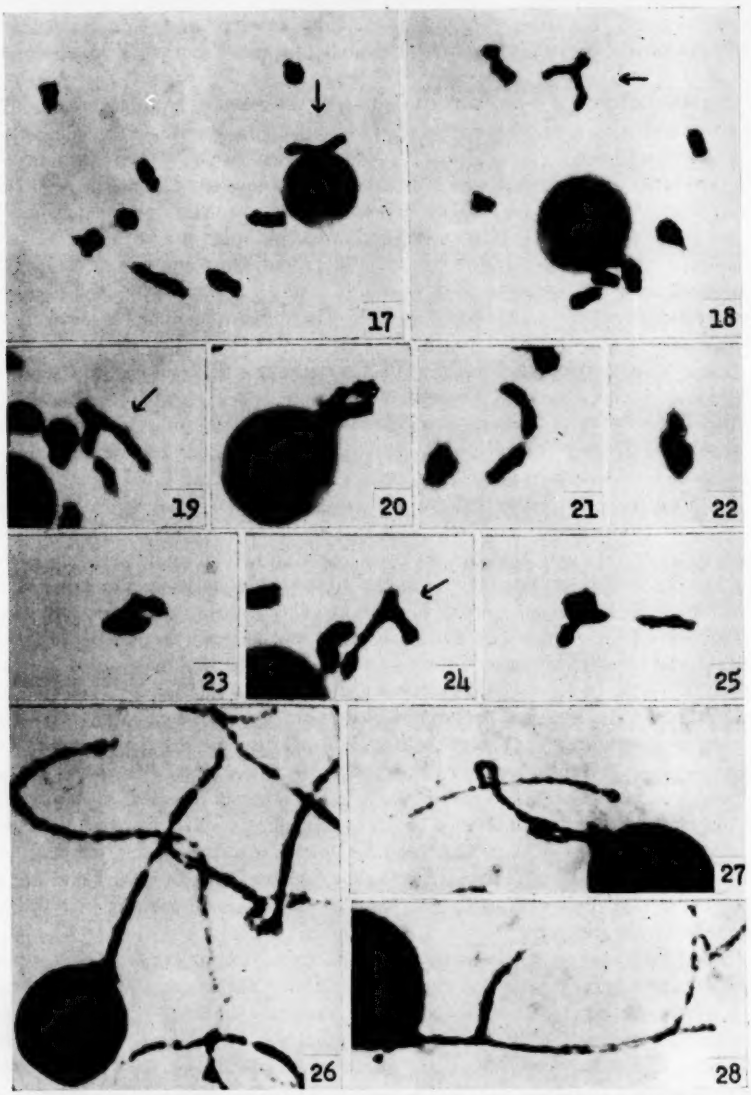
One plant from the ultraviolet treated series possessed a type of chromosomal aberration which was not observed in the 12 bronze plants from the ultraviolet series. This plant exhibited a genetic deficiency for the *R* locus, which was not visible cytologically when heterozygous with a normal chromosome 10. The pollen from this plant was 50% aborted. Its progeny showed no female or male transmission, which would be evidence for a deficiency (r^g) and not a mutation of R^r *K* to R^g *K*. It is important to emphasize the fact that this interstitial deficiency was in compound with a knobless chromosome since a minute interstitial deficiency would not be detected readily in the knobbed chromosome. The aberrant knob on chromosome 10 has a tendency to increase the length of the chromosome by stretching the long arm.

A second type of alteration which did not occur among the bronze plants from the ultraviolet treated series, but did occur in the X-ray treated series of chromosome 9, is the deficiency translocation. Three deficiency translocations were found in the chromosome 10 series in which ultraviolet radiation was used. Two of these translocations were found in r^g plants and one in a variegated plant.

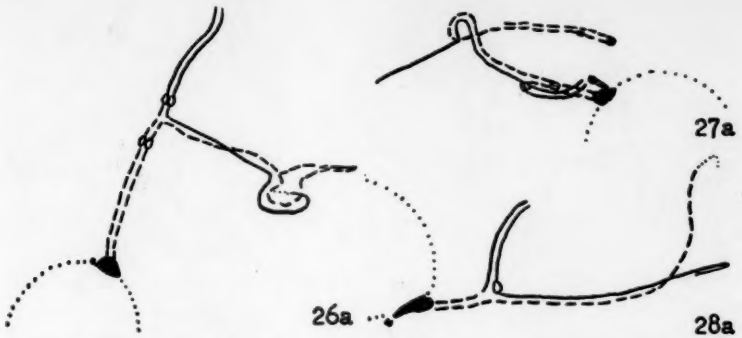
The case from the variegated plant showed a 3-chromosome deficiency translocation at diakinesis and pachytene. One striking difference between this 3-chromosome deficiency translocation and the cases described previously, is the variation in the location of the two centromeres of the dicentric chromosome. The centromere shown in figure 13 is about twice its normal size. This probably represents either two permanently fused centromeres, or two separate centromeres with the orientation of the two chromosomal arms such that the two centromeres had coalesced. Figure 14 shows this same translocation with two widely separated centomeres (indicated by two arrows). The distance between the two centromeres is probably magnified slightly in this figure since one of the centromeres of the dicentic chromosome was adherent to a centromere of another chromosome. This stretched the euchromatin between the two centromeres of the dicentric chromosome. Two additional figures of this translocation were observed at pachytene, one of which shows an enlarged or double centromere and one which shows two separate centromeres; the distance between the two centromeres was similar to the distance shown in figure 14. It would seem likely that these various figures represent the deficiency translocation in different sectors of the tassel.

One of the other two deficiency translocations from r^g plants exhibited three associated chromosomes at diakinesis. Figure 15 illustrates this translocation at diakinesis.

A third type of cytological aberration was found among the r^g plants which was not found in either the ultraviolet or X-ray series of chromosome 9. This plant possessed four associated elements at diakinesis and was designated a 3½-chromosome deficiency translocation. At diakinesis this r^g plant exhibited consistently a chain of four chromosomes and associations with three chiasmata (fig. 16). The following observations were made at pachytene: (1) chromosome 10 and also either chromosome 2 or 4 were involved in some type of translocation (the actual union was not observed); (2) normal chromosome 10 was synapsed with a chromosome 10



FIGURES 17–18.—(× 1600). Diakinesis cells with 3-chromosome deficiency translocations (indicated by arrows) and eight bivalents. FIGURES 19–25.—(× 2600) 3-chromosome deficiency translocations at diakinesis. FIGURES 26–28.—(× 1300) 3-chromosome deficiency translocations between chromosomes 6 and 10 at pachytene; see diagrams.



FIGURES 26a–28a.—Diagrams of figures 26–28. In each diagram the normal chromosome 10 is represented by a solid line; the translocated chromosome is illustrated by partly solid and broken lines; the normal chromosome 6 is represented by broken lines.

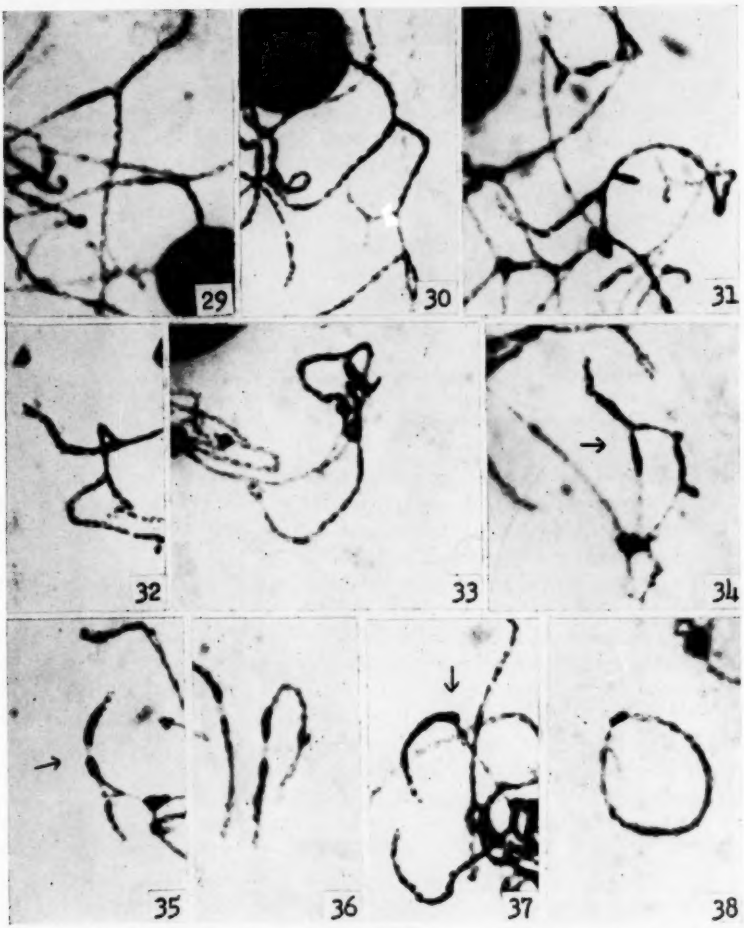
which was a great deal longer than normal; (3) one chromosome 2 or 4 was deficient; (4) normal chromosome 2 or 4 was associated with some chromosome other than the deficient one. Presumably, chromosome 10, and either chromosomes 2 or 4, were part of the same translocation.

The cytological analysis showed that nine of the green plants possessed a normal chromosome ten with the aberrant knob as did five of the plant losses observed in the control pollinations. In addition to these five plant losses in the control, two unstable plant mutants and one possible mutant which was intermediate for plant color were found. Thus the nine plant mutants from the ultraviolet treated series cannot be attributed to the treatment since the frequency of these is not significantly different from that in the control. The self pollinated ears from eight of the plants in the U. V. experiment indicated the mutation of R^r K to R^g K and not to r^g, which would be evidence for a deficiency. The ninth plant gave no progeny. The treated male stock was well marked against contamination. The genotype of the treated male parent was g R^r K/g R^r K, *pr pr*, *Pl Pl*. The cytological examination verified the presence of the knob in each of the nine cases. In eight of the mutants from the ultraviolet treated series, *pr* and *g* were confirmed. The presence of *Pl* has been verified in six of the eight mutants; the remaining two cases are being tested. Each of the eight cases from the control have shown the presence of knob 10 and *pr* in their progeny; the *g* and *Pl* contamination markers need yet to be verified.

Ten of the 29 r^g plants were identified as haploids at diakinesis. These haploids were not analyzed further except for verification of the haploidy at anaphase II where the 10 monads are distributed irregularly to the telophase poles.

Effects of X-ray radiation on chromosome 10

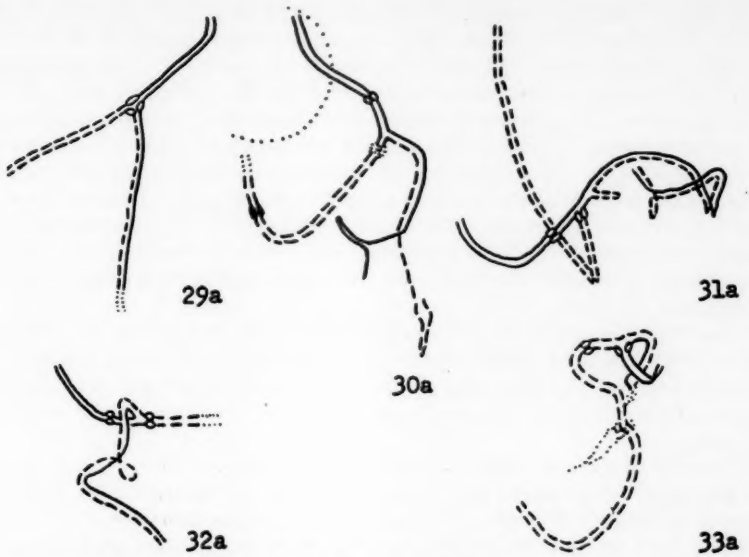
Mature pollen from plants homozygous for the knob and the dominant allele of r^g (R^r K/R^r K) was treated with 400r units of X-radiation and placed on silks of



FIGURES 29–38.—(× 1300). FIGURES 29–33.—3-chromosome deficiency translocations involving chromosome 10 at pachytene; see diagrams. FIGURES 34–37.—Illustrations of a possible deficiency inversion for chromosome 10. FIGURE 38.—A ring chromosome for knobbed-10 chromosome.

homozygous recessive plants, $r^g r^g$. Thirty-nine r^g seedlings were found among 4272 F_1 seedlings, and 29 of these were analyzed cytologically. Six of the r^g plants died in the seedling stage and four gave poor cytological specimens. In addition to the 39 r^g seedlings, three variegated plants were found. Two of these three plants were analyzed. The third case died in the seedling stage. A summary of the cytological results of the 29 r^g plants and the two variegated plants is shown in table 2.

One interstitial deficiency, but no terminal deficiencies, were found among the 29



FIGURES 29a–33a.—Diagrams of figures 29–33. In each diagram the normal chromosome 10 is drawn as a solid line. The translocated chromosome is illustrated by partly broken and solid lines; the other normal chromosome is represented by a broken line.

r^g seedlings. This deficiency involved both the plant and seed color determiners of the R^r locus. This deficiency, which possessed the knob, was invisible cytologically in compound with a knobless chromosome 10. The deficiency showed approximately six percent female transmission. It is now being tested in compound with another knobbed-10 chromosome.

Eight 3-chromosome deficiency translocations were identified in the 29 r^g plants and one in a variegated plant of the X-rayed series. The 3½-chromosome type of deficiency translocation was absent in the X-rayed series of chromosome 10.

In figures 17 to 25, the eight deficiency translocations from the r^g plants and the one from the variegated plant are illustrated in the diakinesis stage. Seven of the eight 3-chromosome deficiency translocations and the translocation in the variegated plant were analyzed in the pachytene stage. In two plants, the 3-chromosome deficiency translocations occurred between chromosome 6, the nucleolar organizer chromosome, and chromosome 10. The dicentric chromosome in both translocations was formed by the fusion of at least the short arm of chromosome 6 and its centromere with the short arm of chromosome 10 and its centromere. In both cases a strand of euchromatin was located between the two centromeres of the dicentric chromosome. It is difficult to determine the origin of this euchromatin since only a limited number of pachytene figures was observed. In figure 26 the long arms of chromosomes 6 and 10 are paired with the thread of euchromatin between the two cen-

tromeres. A pachytene figure from the other plant is shown in figures 27 and 28. In figure 27 the dicentric chromosome is paired homologously with the short arms of chromosomes 10 and 6. The normal long arm of chromosome 6 is synapsed in part with the euchromatin between the two centromeres of the dicentric chromosome. The remainder of the long arms of chromosomes 10 and 6 is paired non-homologously.

In two other plants, one of which was the variegated plant, chromosome 10 and 1 were involved in a 3-chromosome deficiency translocation. The translocation in the r^g plant is shown at pachytene in figure 29. In this particular figure only one enlarged centromere is present. Three additional figures of this translocation were found at pachytene in which two exhibited two separate centromeres and one showed an enlarged centromere. One figure from the variegated plant showed one enlarged centromere.

One r^g plant exhibited a 3-chromosome deficiency translocation of chromosome 10 and 4. In this translocation the short arm of chromosome 10 with its centromere and the long arm of chromosome 4 with its centromere had united to form a dicentric chromosome. A thread of euchromatin was found between the two centromeres.

Figure 30 illustrates a 3-chromosome deficiency translocation between chromosome 10 and either chromosomes 2 or 4. The dicentric chromosome consisted of the short arm of chromosome 10 plus its centromere and long arm of chromosome 2 or 4 plus its centromere. In figure 30, the long arm of normal 10 and the short arm of chromosome 2 or 4 are paired with the euchromatin between the two centromeres; however, this could be non-homologous pairing. The two remaining deficiency translocations are illustrated at pachytene in figures 31 to 33. Figures 31 and 32 are two photos from the same plant. In both of these figures, only the long arm of chromosome 10 is paired with the euchromatin between the two centromeres of the dicentric chromosome. Figure 33 illustrates a 3-chromosome deficiency translocation between chromosome 10 and an unidentified chromosome. In this figure, which clearly shows the two centromeres, only the unidentified chromosome is paired with this region between the two centromeres.

One of the 29 r^g plants possessed an alteration that could be interpreted as a deficiency inversion. Three types of chromosome 10's were observed. One type is represented by figures 34 and 35 in which a knob 10-like substance is now located adjacent to the centromere; normally knob 10 is located near the end of the long arm. These figures show the knob-like substance and the two unpaired long arms of chromosome 10. In the second type, both of the long arms are paired (fig. 36); however, the typical inverted type of pairing was not observed. The third type of chromosome 10 which was observed is illustrated in figure 37. In this type the entire strand of euchromatin distal to the knob is deficient. The pollen from this plant showed 50% abortion.

Ten of the 29 r^g plants were identified as haploid plants from diakinesis and anaphase cells. In each haploid plant, 10 univalents were present.

In one plant the entire irradiated chromosome 10 was deficient. Although no one figure was observed in which each of the 10 chromosomes was isolated, a sufficient number of pachytene stages was examined to verify the absence of even a minute fragment. The examination of the metaphase stage showed also the absence of even

a minute fragment. The diakinesis phase is not a critical stage to determine the absence of minute fragments since small fragments become quite achromatic at this phase.

One of the 29 r^g plants possessed a small fragment or ring chromosome at pachytene. This fragment included about one eighth of the original chromosome 10.

Two ring chromosomes were found in two r^g plants, and one ring chromosome, not deficient for the R^r locus, was included in a variegated plant. The two ring chromosomes from the r^g plants included approximately one fourth and two thirds of the original chromosome 10. The ring from the variegated plant included nearly the entire knobbed-10 chromosome (fig. 38). The original breakage points must have occurred near the end of the short arm of chromosome 10 and in the knob itself.

Five of the 29 plant losses possessed a normal chromosome 10. Four of these cases represented conclusively a plant color mutation of R^r *K* to R^g *K*. The presence of *Pl*, *pr*, and *g* contamination markers has been verified in the four plants. In the fifth plant, *pr* and *Pl* have been identified, but the presence of *g* needs yet to be verified.

DISCUSSION

It is difficult to make a quantitative analysis of the effects of ultraviolet radiation and X-rays on chromosomal aberrations, since there is no direct method of measuring comparable doses. A comparison could be made in terms of some genic or chromosomal equivalent. STADLER (1941) determined the relative effectiveness of these two types of radiation on gene mutation in which he was employing doses equal in their effect on specific gene markers in the endosperm. In another comparative mutation experiment (STADLER 1941), the number of plants with segregating pollen was used as an equivalent for determining comparable doses of X-ray and ultraviolet radiation. If a specific equivalent is selected as the basis for the determination of comparable doses, a major difficulty is encountered immediately. The doses of ultraviolet and X-rays assumed to be comparable in their effects on one type of genic or chromosomal standard, may not be equivalent if another standard is selected. In view of this difficulty, only general inferences can be drawn from the cytological results reported here.

Previously, it has been suggested (STADLER 1941) that there may be a qualitative difference in the type of chromosomal aberration which has been produced in plants following treatment with X-ray and ultraviolet radiation. The writer's data show no gross qualitative difference between effects of the two types of radiation. If each kind of aberration in plants from the ultraviolet treated series is compared with those found in the X-ray treated series, it is found ring chromosomes and a questionable deficiency inversion are the only types of aberrations absent in plants following ultraviolet treatment. Both X-ray and ultraviolet treated series included terminal deficiencies, interstitial deficiencies, fragmented chromosomes and deficiency translocations.

There is indication, however, that a quantitative difference exists between the two types of radiation in the frequency distribution of chromosomal aberrations produced. It is reasonable to assume that if the over-all effect of X-rays is greater than that of ultraviolet radiation in the frequency of production of chromosomal

aberrations at the dosage levels used, ultraviolet rays should be less effective than X-rays in the production of any one specific type of chromosomal alteration. In the paragraphs to follow, it will be shown that a low dose of X-rays (400r) is much more effective than the two highest doses of ultraviolet radiation (U.V. 20″ and 30″) on the frequency of total number of chromosomal aberrations. But the total frequency of terminal deficiencies following ultraviolet treatment (U.V. 20″ and 30″) is greater than that produced by X-rays (400r).

The Chi square method of measuring whether the observed ratio of chromosomal aberration fits with the expected ratio has been used to determine the relative frequency of production of chromosomal alterations following a low dose of X-rays (400r) as compared with the two highest doses of ultraviolet rays (U.V. 20″ and 30″). The following data include the simple and complex deficiencies from both of the chromosomes 9 and 10 series:

	Total population	Total no. cases	Total cases expected
U.V. (20″ and 30″)	8834	14	21.7
X-ray (400r)	6208	23	15.3
Total	15,042	37	37.0

Mean value 37/15,042 = 0.002460

Total (sum of d^2/e) = 6.60 = χ^2

P = 0.01–0.02

The results show a larger number of aberrations following X-ray treatment of 400r than following treatment with ultraviolet radiation for 20 and 30 seconds. This conclusion, however, is based entirely on the assumption of random distribution of chromosomal alterations in the two populations.

The following data show that ultraviolet radiation, though less effective on the over-all frequency of chromosomal aberrations, has a greater effect than X-rays on the frequency of terminal deficiencies. This conclusion is based on results from chromosome 9 series only since terminal deficiencies are questionable in the chromosome 10 series.

	Total pop.	Total df. obs.	% Total df.	Term df. obs.	Term. df. exp.	Complex df. obs.	Complex df. exp.
U.V. (all)	6168	9	32.14	8	3.21	1	5.79
X-ray (all)	3006	19	67.86	2	6.79	17	12.21
Total	9174	28	100.00	10	10.00	18	18.00

Tests of significance of these ratios have been estimated from a statistical table prepared by STEVENS (1942). This table is used for the determination of the limits of expectation where small numbers are involved. The following probabilities have been found from the above ratios:

	Exp. (=n)	Obs.	$p = n/N$	Probability
Complex df.—U.V.	5.79	1	0	.005 × 2 = 0.010
Terminal df.—U.V.	3.21	8	0	.025 × 2 = 0.05

The data show a lower frequency of complex deficiencies in the ultraviolet series than in the X-rayed series. The results indicate also a probably significantly larger number of terminal deficiencies following ultraviolet treatments than in the X-ray treated series.

The low frequency of deficiencies in plants following treatment with ultraviolet radiation could be ascribed to the restitution of the fractured chromosomes at the point of original breakage. SCHULTZ (1951), studying the effects of ultraviolet radiation on a ring chromosome in *Zea mays*, attributed the high frequency of ring loss following treatment with ultraviolet to restitution. He believed that sister strands reunite to form interlocked or double rings which are either lost or included both in one daughter cell.

It could always be argued that the doses of X-ray and ultraviolet radiation were not strictly comparable in the present experiment. STADLER (1938) showed that the low rate of deficiencies in plants treated with ultraviolet was not a function of the low dosage. He compared the number of plants showing pollen abortion in the X-ray treated series with those in the ultraviolet treated series. The doses of X-rays used included 150r, 600r, 2400r in one series and 1450r in another series. The dose of ultraviolet used was stated as being near the tolerance limit for the wave length. He found consistently a higher frequency of plants with aborted pollen in the 600r, 1450r, and 2400r X-rayed series than in plants which were treated with a heavy dose of ultraviolet radiation. STADLER concluded that a qualitative difference exists between the two types of radiation.

The low frequency of complex aberrations in the ultraviolet series could be due to a low rate of coincident breaks. An alternative hypothesis to this would be that ultraviolet radiation produces as many breaks per nucleus as X-rays but the broken chromosomal ends do not reattach. If this were the case, there should be numerous broken chromosomes in single cells. These coincident losses were not analyzed in the cytological material examined in the present investigation.

SUMMARY

A cytological analysis was made of the effects of ultraviolet radiation and X-rays on chromosomes 9 and 10 of *Zea mays*. A stock homozygous recessive for the gene *bz* in the short arm of chromosome 9, and a stock with the gene r^g in the long arm of chromosome 10 were pollinated by irradiated pollen from stocks homozygous dominant for the two marker genes and the knob. The F_1 plants which exhibited the recessive phenotype for the specific gene markers on chromosomes 9 and 10 were saved for cytological analysis.

Analysis of the chromosomal aberrations showed no gross qualitative difference between the effects of ultraviolet and X-ray radiation. However, a quantitative difference was found between the two types of radiation in the frequency distribution of chromosomal alterations. A lower frequency of complex deficiencies and higher frequency of terminal deficiencies occurred in chromosome 9 following treatment with ultraviolet radiation (U.V. 10, 20, and 30 seconds) than in chromosome 9 following X-ray treatments (400r, 800r, and 1000r). It could not be determined whether this was due to (1) a low rate of coincident breaks, or (2) the same frequency of

breaks in ultraviolet and X-ray series but failure of fusion of broken chromosomal ends in the ultraviolet series.

ACKNOWLEDGMENTS

To the late DR. L. J. STADLER I wish to acknowledge gratefully the stimulating guidance, encouragement, and advice given by him throughout the course of this investigation. I wish to thank DRS. J. R. SINGLETON, HARRIET B. CREIGHTON, E. R. SEARS, E. NOVITSKI, and J. R. LAUGHNAN for their suggestions and helpful criticisms during the preparation of this manuscript.

LITERATURE CITED

DE BOER, KATHERINE O., 1945 Comparison of ultra-violet and X-ray deficiencies. Maize Genetics Coop. News Letter, Cornell Univ. 43–44.

DOLLINGER, E. J., 1954 Studies on induced mutation in maize. Genetics **39:** 750–766.

LONGLEY, A. E., 1938 Abnormal segregation during megasporogenesis in maize. J. Agr. Research **56:** 117–195.

MCCLINTOCK, BARBARA, 1931 Cytological observations of deficiencies involving known genes, translocations and an inversion in *Zea mays*. Missouri Agric. Expt. Sta. Res. Bull. **163:** 1–30.

SCHULTZ, J., 1951 The effect of ultra-violet radiation on a ring chromosome in *Zea mays*. Proc. Nat. Acad. Sci. U. S. **37:** 590–600.

SINGLETON, W. R., 1939 Cytological observations on deficiencies produced by treating maize pollen with ultra-violet light. Genetics **24:** 109.

SINGLETON, W. R., and F. J. CLARK, 1940 Cytological effects of treating maize pollen with ultra-violet light. Genetics **25:** 136.

STADLER, L. J., 1938 Comparative effects of X-rays and ultra-violet on translocation. Annual Report of Coop. Corn Investigations, Missouri Agricultural Experiment Station 140–154.

1939 Genetic studies with ultra-violet radiation. Proc. VII Inter. Congr. Genet. 269–276.

1941 The comparison of ultra-violet and X-ray effects on mutation. Cold Spring Harbor Symposia Quant. Biol. **9:** 168–177.

STADLER, L. J., and H. ROMAN, 1943 The genetic nature of X-ray and ultra-violet induced mutations affecting the gene *A* in maize. Genetics **28:** 91.

1948 The effect of X-rays upon mutation of the gene *A* in maize. Genetics **33:** 273–303.

STADLER, L. J., and G. F. SPRAGUE, 1937 Contrasts in the genetic effects of ultra-violet radiation and X-rays. Science **85:** 57–58.

STADLER, L. J., and C. SWANSON, 1951 The Genetic Effects of Ultra-violet Radiations. *Radiation Biology*, Vol. II. McGraw Hill Book Co. (in press).

STADLER, L. J., and F. M. UBER, 1942 Genetic effects of ultra-violet radiation in maize. IV. Comparison of monochromatic radiation. Genetics **27:** 84–118.

STEVENS, W. L., 1942 Accuracy of mutation rates. J. Genet. **43:** 301–307.

CAROTENOIDS OF THE APRICOT TOMATO AND ITS HYBRIDS WITH YELLOW AND TANGERINE

J. A. JENKINS AND G. MACKINNEY

Departments of Genetics and Food Technology, University of California, Berkeley

Received March 4, 1955

THE red color of the cultivated tomato, *Lycopersicon esculentum* Mill. is due to the carotenoid lycopene. LEROSEN *et al.* (1941) and ZECHMEISTER *et al.* (1941) first analyzed the carotenoids of yellow (*rr*) and tangerine (*tt*) strains, each of which differs from the red by a single recessive gene. MACKINNEY and JENKINS (1952), JENKINS and MACKINNEY (1953), and TOMES *et al.* (1953) have examined the yellow-tangerine double recessive. LINCOLN and PORTER (1950) transferred from *L. hirsutum* a gene (*B*), which, when homozygous in *L. esculentum*, resulted in a type described as beta orange. TOMES *et al.* (1953) reported further on hybrids involving beta orange. More recently TOMES and QUACKENBUSH (1953) have suggested that the intermediate appearance of the B^+B heterozygotes is really due to the presence of a dominant inhibitor derived from the red-fruited parent. That is, *B* is completely dominant to its allele (B^+) in cultivated tomatoes. MACKINNEY, RICK and JENKINS (1954) reported a gene similar to *B* in a variety of *L. pimpinellifolium* from the Galapagos Islands.

In this paper we discuss the effects of a new recessive gene, apricot (*at*), which is involved in the biosynthesis of carotenoids. In particular, we describe the carotenoids of the apricot homozygote (*atat*) together with the two double recessives, yellow-apricot (*rr atat*) and tangerine-apricot (*tt atat*).

MATERIAL AND METHODS

The apricot tomato was found in the Tehuacan market, State of Puebla, Mexico in December 1945. It was somewhat more orange in color than a typical yellow acquired on the same day. The line established from the original apricot tomato has oval-shaped two-loculed fruits averaging about 45g in weight. Characteristically the flesh has a few small red spots visible from the surface as well as a pink blush in the interior locular walls near the placenta. Because yellow tomatoes vary considerably in color, the overall appearance of some yellows and apricots may be quite similar. However, the yellows never have the small islands of sharply defined red tissue nor do they have the pink locular wall. Where pinkish regions occur in yellow tomatoes, they are confined to the outer walls and, for the most part, toward the base of the fruit.

Apricot was hybridized with all of the lines listed in table 1. All crosses were made in an insect-free greenhouse where all F_1 cultures were grown to maturity. For the most part, F_2 and all other segregating generations were started in the greenhouse and transferred to the field. Where technical color names were used they were taken from RIDGWAY (1912). Carotenoid analyses were made from fruits of individual plants according to methods described earlier (MACKINNEY and JENKINS 1949, 1952).

TABLE 1

Description of the tomato lines

Line	Genotype	Source
Red	Colorless skin (*yy*).	JENKINS
Yellow	Dwarf (*dd*), Pubescent fruits (*pp*), Ovate fruits (*oo*), Compound inflorescence (*ss*), Yellow flesh (*rr*).	LINDSTROM *via* RICK
Tangerine	Colorless skin (*yy*), Uniform fruit (*uu*), Tangerine flesh (*tt*).	MACARTHUR *via* LESLEY
Beta Orange	High β-carotene (BB)	PORTER
Apricot	Colorless skin (*yy*), Apricot flesh (*atat*).	JENKINS
Yellow-tangerine	Yellow flesh (*rr*), Tangerine flesh (*tt*)	JENKINS

TABLE 2

Carotenoid contents of homozygous lines, in micrograms per gram of fresh fruit

Line	Xanthophylls	Lycopene* Isomers	ζ-carotene	β-carotene	Phytofluene
Yellow	3–5	0–1	—	1–3	0.1
Apricot	3–7	2–5**	—	6–10	0.2
Yellow-apricot	6.2	—	—	1.2	trace
	5.4	—	—	0.4	0.5
	5.7	—	—	0.4	trace
Tangerine-apricot	negligible	15.0	0.7	1.8	0.5
	negligible	6.5	1.0	1.0	0.6
	negligible	14.0	0.8	0.7	0.7
	negligible	21.4	0.6	2.1	1.1
Yellow-tangerine	negligible	10–15	0.1	0.5–1.0	0.7–1.0

* All-*trans* lycopene for yellow and apricot, mostly prolycopene for tangerine-apricot and yellow-tangerine.

** In a seedless specimen, this rose to 23 μg.

RESULTS

Carotenoids of apricot

The apricot line not only resembles many yellows in general appearance but has very nearly the same carotenoids (table 2). Both have essentially the same content of xanthophylls and of phytofluene. Though apricot has a somewhat higher lycopene content, the major difference between the two lies in the higher content of β-carotene of the apricot, which is at a level typical of normal red-fruited tomatoes.

First generation hybrids

The F_1 hybrids red $\times$ apricot, yellow $\times$ apricot, yellow-tangerine $\times$ apricot and tangerine $\times$ apricot were red in color, but as can be seen from table 3, the lycopene content was not always within the range of a typical red, which normally has 70 to

TABLE 3

*Carotenoid contents of the F_1 hybrids with Apricot, in micrograms per gram of fresh fruit**

F_1 Hybrid	Lycopene	β-carotene	Phytofluene
Apricot × red	67.5	8.0	6.8
Yellow × apricot	73.9	8.7	4.6
Yellow-tangerine × apricot**	61.0	7.6	4.7
Tangerine × apricot	67.0	7.0	not determined
Beta Orange × Apricot	50.0	54.6	15.4

* All hybrids had in addition to the listed carotenoids traces of γ-carotene.

** traces of ζ-carotene.

130 μg of lycopene per gram of fresh fruit. However, these small deviations from the normal red carotenoid content could very well have been due to environmental effects, since data obtained in 1953 varied somewhat from the analyses of 1954. In the case of beta orange × apricot the F_1 color was red-orange (grenadine red to flame scarlet) and had about equal proportions of lycopene and β-carotene together with a high phytofluene content (table 3). In its ratio of β-carotene to lycopene our beta orange × apricot F_1 was similar to the F_1 red × beta orange reported by LINCOLN and PORTER (1950). That is, the characteristic carotenoid content of F_1 beta orange × apricot apparently depends upon heterozygosity at the *B* locus rather than heterozygosity at the *at* locus. Data from all of the F_1 hybrids consistently show that apricot is a new recessive gene that is not allelic to any of the carotenoid genes already described.

Segregating generations of the yellow × apricot hybrid

In a total of 139 F_2 plants, belonging to 4 different cultures, 80 were red and 59 non-red, which does not differ significantly from a 9:7 ratio expected from segregation of two independent loci. Data from backcrosses of F_1 to both parents were in full agreement. The backcross to yellow had 32 red and 26 yellow, while the backcross to apricot gave 42 red and 27 apricot. Neither of the backcross ratios differ significantly from the 1:1 expectation.

The non-red plants obtained in the F_2 ranged continuously from a fully colored apricot to a light yellow. Since the double recessive yellow-apricot (*rr atat*) could not be recognized in F_2, selfed progeny of non-red plants in the F_2 as well as in both backcrosses were grown. In order to avoid contamination from foreign pollen only plants growing in the greenhouse were chosen as parents of the selfed progenies.

One F_3 culture (534H129) was obtained that segregated 14 apricot plants to 4 plants with lighter colored fruits that lacked the red areas near the placenta, which are characteristic of apricot. The F_4 progenies that were grown from these four F_3 plants likewise were lighter in color and lacked the red placental areas. One of the F_3 plants (534H129.1) when test-crossed with yellow gave only yellow progeny, indicating that the F_3 plant was homozygous for yellow. In addition, two of its F_4 progeny were test-crossed with apricot and the test-cross progeny were all apricot. Consequently the line established from 534H129.1 was the double recessive yellow-apricot.

The yellow-apricot was also recovered from selfed progeny of the F_1 backcrossed to apricot. Progenies were grown from 5 apricot plants, which were expected to be *r*$^+$*r*$^+$ *atat* or *r*$^+$*r atat*. One progeny gave only pure apricot plants, while four of them segregated giving a total of 26 apricot to 8 yellow-apricot. The latter were classified as the double recessive because they lacked the pink blush on the transverse locular walls adjacent to the placenta, which is characteristic of apricot fruit. Furthermore, one of the 26 apricot plants was test-crossed with both parents: with apricot the progeny were all apricot and with yellow the test-cross progeny had 5 red and 5 yellow plants. Therefore the tested plant had the constitution *r*$^+$*r atat*. On selfing it gave 7 apricot to 3 yellow-apricot.

Recognition of the yellow-apricot in the selfed progenies of the backcross of F_1 to yellow was much more difficult. However, of the 4 yellow plants of the first backcross generation that were progeny tested, one gave progeny that segregated (2 yellow : 4 yellow-apricot). On the other hand, another of the first backcross yellow plants was shown to have the genotype *rr at*$^+$*at* by testcrosses yet no yellow-apricot plants were detected in a selfed progeny of 10 individuals.

The pigment contents of 3 yellow-apricot (*rr atat*) plants are given in table 2; the first two are F_4 while the last is a third generation of the backcross to apricot. As was to be expected from their light color, they contained very little pigment, mostly xanthophylls, while no lycopene was detected and the β-carotene content was frequently somewhat lower than in a typical yellow. In other words, the yellow-apricot might be characterized as a weakly-pigmented yellow.

Segregating generations of the tangerine × apricot hybrid

The F_2 tangerine × apricot segregated 41 red to 34 non-red, which does not differ significantly from a 9:7 expectation on the basis of two independently segregating genes. However, as in the case of the yellow × apricot, the non-red plants could not be further classified with any degree of certainty. The main difficulty was due to environmental variability which tended to obliterate discontinuities between the various color classes. Even in F_3 cultures from individual non-red F_2 plants, classification was not satisfactory. A further complication arose. Since nearly 3 percent of the F_3 plants were red, these undoubtedly arose from natural crossing between F_2 plants, which were grown in the field.

A careful comparison of cut fruits of F_3 plants showed that there was a type that did not resemble either tangerine or apricot. This intermediate type had a more pinkish color in the central part of the fruit and the outer wall was less pigmented than in the tangerine. The cut section of the outer wall was not very different from apricot. Selfed progenies were grown in the greenhouse from individual F_3 plants of the various color types including the new intermediate. At least one plant in each F_4 progeny was test-crossed to both parents, also selfed seeds (F_5) were collected from each of the test-crossed F_4 plants. Eight of the latter gave only all tangerine or all apricot progeny depending upon the parent used. Therefore, these 8 F_4 plants were tangerine-apricot (*tt atat*).

The carotenoid contents of four different tangerine-apricot plants are given in table 2 together with that of the yellow-tangerine. Tangerine-apricot is intermediate

between tangerine and apricot in its total content, but in characteristic constituents, principally prolycopene, it may be regarded as a weak tangerine. However, the decrease in the various pigments has not been proportional. Of the various carotenoids of tangerine, poly-*cis* ψ-carotene was not detected in tangerine-apricot, ζ-carotene showed the most marked decrease, followed in order by phytofluene, β-carotene and finally the lycopene set.

DISCUSSION

The apricot locus does not appear to be closely linked with any of the major carotenoid loci, nor were there any close linkages between apricot and any of the marker genes segregating in the various stocks. However, with the large number of small cultures examined, other than close linkages could be easily overlooked.

In its main effect on the suppression of lycopene, apricot acts very much like the yellow gene. Both have a very similar effect when they are crossed with tangerine, the carotenoids of the lycopene set are decreased in amount and the main constituent is prolycopene, the characteristic carotenoid of the tangerine. In the yellow-apricot, where both genes are homozygous, their separate actions on the suppression of lycopene are reinforced—we did not detect lycopene in yellow-apricot. On the other hand, the lycopene that is formed in yellow and in apricot is found in quite different regions of the fruit.

The action of the yellow and apricot genes on β-carotene synthesis is also quite different. Using the red as a standard, *at* does not have any effect on β-carotene, while yellow does. When the two are together in yellow-apricot, the inhibiting effect of *r* is enhanced. Again using the red as a standard, neigher *t* nor *at* by themselves have any appreciable effect on β-carotene, but when together in the tangerine-apricot, they have nearly as marked effect in reducing β-carotene as does the combination yellow-apricot.

Thus, while the apricot and yellow genes have many points of similarity in their action, they are distinct both in location and in their effect on carotenoid synthesis. Unfortunately at this time we cannot do more than describe the effects of these genes in their various combinations on the many carotenoid pigments. With the possible exception of the *B* gene, the effects are manifold in that each gene alters the content of more than one pigment and in a complex fashion.

SUMMARY

A new recessive gene apricot affecting the carotenoids of the tomato is described. The carotenoid contents of homozygous apricot (*at at*) and the two double recessives yellow-apricot (*rr at at*) and tangerine-apricot (*tt at at*) are given. The effects of the apricot gene are similar to those of yellow but there are distinct differences.

ACKNOWLEDGMENT

Acknowledgment is made of a National Science Foundation grant to G. MACKINNEY. Collections of Mexican tomatoes were made by J. A. JENKINS while holding a John Simon Guggenheim Memorial Fellowship.

LITERATURE CITED

JENKINS, J. A., and G. MACKINNEY, 1953 Inheritance of carotenoid differences in the tomato hybrid yellow × tangerine. Genetics **38:** 107–116.

LEROSEN, A. L., F. W. WENT, and L. ZECHMEISTER, 1941 Relation between genes and carotenoids of the tomato. Proc. Nat. Acad. Sci. U. S. **27:** 236–242.

LINCOLN, R. E., and J. W. PORTER, 1950 Inheritance of *beta*-carotene in tomatoes. Genetics **35:** 206–211.

MACKINNEY, G., and J. A. JENKINS, 1949 Inheritance of carotenoid differences in *Lycopersicon esculentum* strains. Proc. Nat. Acad. Sci. U. S. **35:** 284–291.

1952 Carotenoid differences in tomatoes. Proc. Nat. Acad. Sci. U. S. **38:** 48–52.

MACKINNEY, G., C. M. RICK, and J. A. JENKINS, 1954 Carotenoid differences in Lycopersicon: Hybrids of an unusual race of *L. pimpinellifolium*. Proc. Nat. Acad. Sci. U. S. **40:** 695–699.

RIDGWAY, R., 1912 *Color Standards and Color Nomenclature.* Washington, D. C. 43 p., pl. I–LIII.

TOMES, M. L., and F. W. QUACKENBUSH, 1953 Two factor interaction governing beta-carotene content in the tomato. Genetics **38:** 696–697.

TOMES, M. L., F. W. QUACKENBUSH, O. E. NELSON, JR., and BETTY NORTH, 1953 The inheritance of carotenoid pigment systems in the tomato. Genetics **38:** 117–127.

ZECHMEISTER, L., A. L. LEROSEN, F. W. WENT, and L. PAULING, 1941 Prolycopene, a naturally occurring stereoisomer of lycopene. Proc. Nat. Acad. Sci. U. S. **27:** 468–474.

GENETIC MECHANISMS GOVERNING THE EFFECT OF CANAVANINE ON NEUROSPORA CRASSA

WILLIAM R. LOCKHART[1] AND HAROLD R. GARNER[2]

Department of Bacteriology, Iowa State College, Ames, Iowa and Department of Biological Sciences, Purdue University, Lafayette, Indiana

Received March 7, 1955

HOROWITZ and SRB (1948) first noted that wild type strains of *Neurospora crassa* may be sensitive to the amino acid canavanine. They reported three types: one sensitive, one resistant, and one of intermediate sensitivity. TEAS (1951) studied two mutant homoserine-requiring strains, 46003-R (canavanine resistant) and 46003-S (sensitive). He found that canavanine sensitivity is heritable and independent of the amino acid requirement. Canavanine inhibition of sensitive strains is competitively relieved by arginine or lysine (HOROWITZ and SRB 1948). Some resistant strains requiring homoserine are able to utilize canavanine instead, and this stimulatory effect is also competitively antagonized by arginine or lysine (TEAS 1951).

These data suggested that resistant strains have the ability to "detoxify" canavanine by converting it to a homoserine-like substance, and that canavanine reaction is governed by a pair of allelic genes. Such an explanation does not account, however, for the existence of strains of intermediate sensitivity. Although one might postulate a third allele, quantitative responses of this type are often the result of gene interaction. With the haploid Neurospora, such interaction would require the participation of two or more nonallelic genes. We have made crosses between resistant and sensitive threonineless strains and screened the progeny for segregation of the canavanine character. The results indicate that the reaction of Neurospora to canavanine is governed by two separate genes.

MATERIALS AND METHODS

The mutant strains of *Neurospora crassa* used in these experiments were supplied by MRS. S. EMERSON and MRS. M. B. MITCHELL. Two of the strains as indicated in table 1 are reisolates which are identical by all biochemical tests to the originals as reported by TEAS (1947). The threonine-less gene in strain 44105 is probably identical to that in strain 46003 (TEAS 1947). MCCLINTOCK (1945) noted a translocation in a strain of 44105. There is no evidence that this is linked with the threonine-less gene in that stock. It is not known whether this translocation is present in the stock used here.

Stock cultures were maintained on slants of complete medium (BEADLE and TATUM 1945). For crosses, a slant of Westergaard medium (WESTERGAARD and

[1] Supported in part by funds from the Industrial Science Research Institute of Iowa State College.

[2] Scholar in Cancer Research of the American Cancer Society.

TABLE 1

Neurospora strains

Strain	Canavanine susceptibility	Amino acid requirement
G2-6A (44105)	sensitive	threonine, isoleucine, α-aminobutyric acid, or homoserine
44104a	resistant	threonine, isoleucine, or α-aminobutyric acid
G147-5a(51504)	resistant	threonine + methionine, or homoserine

MITCHELL 1947), supplemented with 5 mg of D,L-threonine and 1 mg of D,L-methionine per 100 ml, was inoculated with a loopful of dry spores of one of the resistant strains. After 4 days incubation at room temperature, a second inoculation was made with strain G2-6A (44105). After perithecia had formed, ascospore dissections were carried out and individual spores planted on slants of Horowitz' glycerol-tartrate medium (BEADLE and TATUM 1945) supplemented with threonine and methionine.

Growth experiments were carried out in 125 ml erlenmeyer flasks containing 20 ml of minimal medium (BEADLE and TATUM 1945) and supplement. Flasks were inoculated with one drop of a light conidial suspension and incubated 72 hours at room temperature (ca 25°C). Mycelial pads were removed, dried at 85°C, and weighed.

Canavanine flavianate was isolated from jack bean meal by the method of GULLAND and MORRIS (1935) and the flavianate decomposed according to the procedure of CADDEN (1940). The product was used as the sulfate. Other amino acids used were obtained from commercial sources.

RESULTS AND DISCUSSION

In crosses of sensitive strain G2-6A (44105) with resistant strains 44104a and G147-5a (51504), analyzable asci (*ie*, at least one spore recovered from each pair) were obtained showing the following types of segregation of the canavanine character:

RRSS (two asci)
IISS (two asci)
IRSS (one ascus)

(R = resistant; S = sensitive; I = intermediate)

Spores obtained from eleven non-analyzable asci were also tested, but only resistant (R) and sensitive (S) types were obtained. Results of typical growth experiments with the parent strains and with the strains derived from one complete ascus obtained from a cross of strains 44104a and G2-6A (44105) are presented in table 2. Replicate experiments with these and the other strains tested yielded similar results. While resistant strains are not inhibited (and are sometimes even stimulated) by canavanine, strains of intermediate phenotype consistently produce in the presence of canavanine only 50 to 75 per cent of their normal mycelial weight. Sensitive strains, on the other hand, are inhibited completely by concentrations of canavanine much lower than 1.0 mg per flask.

TABLE 2

Growth of various canavanine phenotypes in the presence and absence of canavanine

Strain	Major gene*	Dry wt of mycelium (mg) Supplement per flask of minimal medium		Canavanine susceptibility**
		threonine, 1.0 mg methionine, 0.2 mg	threonine, 1.0 mg methionine, 0.2 mg canavanine, 1.0 mg	
G2-6A	44105	44	0	S
44104a	44104	26	43	R
G147-5a	51504	18	18	R
K16-1***	44104	53	46	I
K16-2	44104	61	37	I
K16-3	44104	58	61	R
K16-4	44104	38	47	R
K16-5	44105	50	0	S
K16-6	44105	44	0	S
K16-7	44105	44	0	S
K16-8	44105	40	0	S

* Refers to amino acid requirement (see table 1).

** R = resistant; S = sensitive, I = intermediate.

*** Cultures K16-1 to K16-8 were derived from a single ascus obtained in a cross between strains G2-6A and 44104a.

It is apparent that the phenotypes R, S and I are not governed by a set of alleles at a single locus, since the intermediate type appears in the progeny of crosses between resistant and sensitive strains. The only new genetic type possible under these circumstances would be a recombination of non-allelic genes present in the parents. Therefore the new intermediate phenotype must contain both the genes which, when alone, determine resistance or sensitivity in the parent strains.

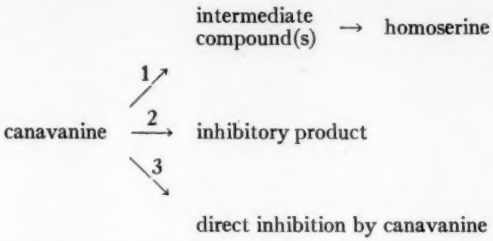
Designating the genes conferring resistance or sensitivity as *R* and *S* respectively, and their alleles as *r* and *s*, the possible segregations (other than simple recovery of the parent types) may be represented as follows:

P_1 *Rs* (resistant) × *rS* (sensitive)

F_1 *Rs RS rS rs* or *RS RS rs rs*

From the phenotypic segregations noted earlier it may be seen that if *Rs* and *rS* are the parent genotypes and if the genotype *RS* is intermediate, the progeny containing the alleles of both the *R* and *S* genes (genotype *rs*) must be sensitive in phenotype.

At the present time the nature of the difference between the *R* and *S* genes and their alleles is not known. Although we have assumed in the discussion which follows that the genes *r* and *s* are simply "inactive", it must be recognized that resistance or sensitivity to a drug can be attained by a variety of mechanisms other than the blocking of a reaction. As the simplest explanation suitable for a working hypothesis, however, the following reaction scheme is tentatively proposed:



Reaction 1 would lead to a homoserine precursor, but not directly to homoserine itself, since not all resistant strains can utilize canavanine in place of homoserine (TEAS 1951). The product of reaction 2 is inhibitory to growth. These alternative reactions would explain TEAS' observation that arginine and lysine can competitively antagonize this substance either in inhibition or in growth promotion. Canavanine may also inhibit directly, or be slowly converted to still another inhibitory product (reaction 3).

Synthesis of the enzyme mediating reaction 1 is controlled by the *R* gene, of the enzyme for reaction 2 by the *S* gene. The respective alleles *r* and *s* are considered inactive, either through inability to elaborate the enzymes or because of a quantitative or qualitative change in the enzymes produced. The genotype *Rs* would be resistant because the reaction product is either inert or promotes growth upon conversion to homoserine (depending on whether there is also present a gene mutation which blocks the homoserine synthesis beyond this point). The genotype *rS*, conversely, would be sensitive. Type *RS* is intermediate in sensitivity, both the inhibitory and stimulatory products being formed from canavanine at about the same rate. The genotype *rs* is sensitive, since reaction 3 (which may be so slow as to be quantitatively insignificant in the presence of reactions 1 or 2) can still take place.

If this is true, two genetically different strains could appear equally sensitive to canavanine. From ascus K16 (table 2), for example, strains 5 and 6 should differ from strains 7 and 8, one pair having the genotype *rs* while the other has the genotype *rS*. Genetic evidence on this point would be essentially negative in character (crosses of the *rs* type with resistant strains should never yield progeny of intermediate sensitivity). However, if sensitivity to canavanine may be the result of two biochemically different processes one would expect to find physiological differences between the two sensitive types. This was confirmed by growth experiments in which these strains were grown in the presence of independently varied concentrations of both canavanine and arginine. The results indicate an arginine:canavanine molar ratio of 3:1 for complete relief of inhibition of strains K16-5 and 6, and a ratio of 7:1 for complete relief with strains K16-7 and 8. If the amino acids compete for different enzymes in the two cases, it is not surprising that different molar ratios are required for competitive relief of inhibition of the two sensitive types. We have also found that strains K16-5 and 6 are sensitive to α-aminobutyric acid at concentrations above .05 mg per flask, while strains K16-7 and 8 are not inhibited even by much higher concentrations of this compound.

There is no evidence that canavanine is a normal intermediate in Neurospora

metabolism; the enzymes which attack it may have as their usual substrate some chemically similar substance. Homoserine and its precursors seem to be involved in these relationships. It should be possible to identify the immediate products of canavanine metabolism by the various genetic types, and thus to help elucidate the metabolic pathways by which homoserine is formed.

SUMMARY

Experiments are reported which support the contention that in Neurospora, resistance and sensitivity to the amino acid canavanine are determined by two nonallelic genes. In crosses between resistant and sensitive strains, types of intermediate sensitivity occur in the progeny. The observed segregations suggest that the intermediate phenotype contains both these genes and that two genetically different sensitive types may occur. A tentative metabolic scheme is presented in explanation of these data.

LITERATURE CITED

BEADLE, G. W., and E. L. TATUM, 1945 Neurospora II. Methods of producing and detecting mutations concerned with nutritional requirements. Am. J. Botany **32:** 678–686.

CADDEN, J. F., 1940 Spatial configuration and preparation of canavanine. Proc. Soc. Exp. Biol. Med. **45:** 224–226.

GULLAND, J. M., and C. J. O. R. MORRIS, 1935 Canavanine. J. Chem. Soc. **1935:** 763–766.

HOROWITZ, N. H., and A. M. SRB, 1948 Growth inhibition of Neurospora by canavanine, and its reversal. J. Biol. Chem. **174:** 371–378.

MCCLINTOCK, BARBARA, 1945 Neurospora I. Preliminary observations of the chromosomes of *Neurospora crassa*. Am. J. Botany **32:** 671–678.

TEAS, H. J., 1947 The biochemistry and genetics of threonine-requiring mutants of *Neurospora crassa*. Ph.D. Thesis, California Institute of Technology.

TEAS, H. J., 1951 Effect of canavanine on mutants of Neurospora and *Bacillus subtilis*. J. Biol. Chem. **190:** 369–375.

WESTERGAARD, M., and H. K. MITCHELL, 1947 Neurospora V. A synthetic medium favoring sexual reproduction. Am. J. Botany **34:** 573–577.

SPONTANEOUS MUTATION IN NON-DIVIDING BACTERIA

FRANCIS J. RYAN

Department of Zoology, Columbia University, New York

Received March 12, 1955

IT has usually been supposed that spontaneous mutations in bacteria take place only among dividing organisms (LURIA and DELBRÜCK 1943). Although NOVICK and SZILARD (1949) showed that in the chemostat the rate of mutation per bacterium per hour could be independent of the rate of growth, the mutations were studied only while the bacteria were dividing. As a consequence of these and other facts it has been proposed that mutations result from rare errors in gene duplication (cf. NEWCOMBE 1953).

When more sensitive methods, to be described in this paper, are brought to bear on the problem, it appears that mutations occurring at a rate of ca. 10^{-9} per bacterium per hour can be detected during the stationary phase. Since this stationary phase does not seem to be dynamic and since the mutations seem to involve the initiation of genotypic changes, it is concluded that mutation can take place in non-dividing bacteria.

MATERIALS AND METHODS

The mutation studied in these experiments was that from a histidineless (h^-) to a non-histidine requiring (h^+) condition in *Escherichia coli* (strain 15). The basic techniques are either described or referred to by RYAN and WAINWRIGHT (1954). Modifications in these procedures will be referred to in relevant places in the text.

RESULTS

The fundamental finding in this investigation was the continued appearance of h^+ mutants in h^- cultures that had ceased multiplying. It has long been appreciated that after a culture of auxotrophs has been plated in agar devoid of the required growth factor, new mutations to prototrophy can take place if the plate allows enough residual growth (RYAN and SCHNEIDER 1949). In this event the final number of prototrophs is not directly proportional to the size of the population introduced into the plate. When the agar and medium do not contain traces of the required growth-factor and appreciable residual growth does not occur, for several days the number of prototrophic colonies is approximately proportional to the total number of bacteria put in the plate (table 1). But with time a few new prototrophic colonies appear which tend to disturb this proportionality by being somewhat too frequent in the lower concentrations. Nonetheless, the number of new prototrophic colonies is usually greater the higher the concentration of parental bacteria.

Under the conditions of the experiments reported in table 1, washed h^- bacteria undergo a negligible residual growth. For example, in six experiments where ca. 5×10^4 washed h^- bacteria were plated on agar medium devoid of histidine (H^-), washed off at different times and replated (RYAN, FRIED and SCHWARTZ 1954), they

TABLE 1

The number of h^+ *mutants found at different times in agar devoid of histidine and inoculated with a series of dilutions of* h^- *cultures*

Dilution	Number of h^+ mutant colonies									
	Experiment (50 hours)					Experiment (180 hours)				
	A	B	C	D	E	A	B	C	D	E
10^0	286	172	134	190	56	288	174	148	194	77
10^{-1}	32	24	18	17	6	34	28	21	21	12
10^{-2}	3	1	1	5	1	3	2	3	9	3
10^{-3}	0	0	1	0	0	1	0	1	1	4
10^{-4}	0	0	1	0	0	2	1	3	0	1
10^{-5}	0	0	0	0	0	0	1	0	0	0
10^{-6}	0	0	0	0	0	0	0	0	0	0

ceased to increase in number after about 24 hours when they had barely doubled (to ca. 1×10^5). This was true whether washed or unwashed agar was employed, although only in recent years has Difco-bacto agar been so clean as to give this result. Furthermore, washed h^- bacteria suspended in liquid H^- medium show no increase in numbers whatsoever, indicating that the very slight residual growth on agar, to a level of ca. 10^5 cells per plate, is not due to the slow synthesis of histidine by h^- bacteria but rather to a very slight contamination of the agar, even when washed (ca. 10^{-1} μg histidine per gram of dry agar). Since the rate of mutation during growth from h^- to h^+ is ca. 3×10^{-8} per bacterium per generation (RYAN and WAINWRIGHT 1954), only one colony per 500 plates could be expected to arise from this cause. Thus, the very slight growth on H^- agar is insufficient to account for the new h^+ colonies found.

Nonetheless, new h^+ colonies continue to appear in appreciable numbers with time on H^- plates as is shown in figure 1. This rise in number cannot be due to contamination during storage, since the colonies are found deep within the agar. It is possible, especially with plates containing large numbers of colonies, that some counted for the first time on the third day were present on the second, but not observed. However, the likelihood of this error can be judged insignificant in view of the fact that h^- colonies found on H^+ agar are usually counted in exactly the same numbers on the second and succeeding days. Furthermore, the new h^+ colonies when first observed are usually smaller than those which were counted the day before.

Before accepting the mutational origin of the new h^+ colonies, not due to residual growth on h^- bacteria on the plate, it is necessary to entertain another possibility. Perhaps the mutations occurred during the growth of the h^- inocula in histidine but, when the h^+ mutants giving rise to the late-appearing colonies were placed on H^- agar devoid of histidine, they grew more slowly than other homogeneous h^+ bacteria and hence formed colonies later (WRIGHT 1953a and b). In this event the new h^+ colonies should exhibit the same sort of clonal variation as do the early-arising h^+ colonies, since in both cases mutations occurring early in the growth of the in-

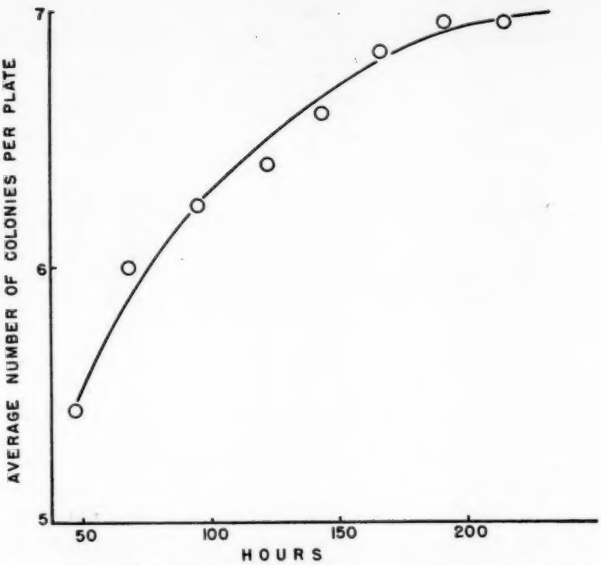


FIGURE 1.—The rise in numbers of h^+ colonies on 25 H^- plates with time. Each plate was inoculated with the whole contents of separate 2 ml cultures containing an average of ca. 3.2×10^7 h^- bacteria. The rate of mutation from h^- to h^+ during growth, calculated from the fraction of plates without colonies at 47 hours, was 3.9×10^{-8} per bacterium per generation.

oculum in histidine would give rise to more mutants than would later-occurring mutations. Table 2 shows that this is not the case. The variances for early-arising h^+ colonies are from 10 to 100 times the mean number of colonies per culture and there are insignificant probabilities that the different cultures were homogeneous with regard to the h^+ mutants they contained. This finding is consistent with the hypothesis that the mutations occurred during the growth of the inoculum in histidine. The late-arising colonies, on the other hand, showed variances approximately equal to the mean numbers and, in most cases, there was a high probability that they formed a homogeneous population. This is inconsistent with the hypothesis that the late-arising h^+ colonies came from h^+ mutants that arose during inoculum growth but grew slowly on the H^- plates.

In experiment 3 of table 2 each of the replicate cultures contained a mixture of h^- *lac*$^-$ (lactose non-fermenting) and h^- *lac*$^+$ bacteria, and the h^+ colonies arising early or late were tested for their *lac* character. The distribution of early-arising *lac*$^+$ and *lac*$^-$ colonies among the different cultures did not fit (χ^2 test; $P = <0.01$) that expected on the hypothesis that each had a chance of being *lac*$^-$ or *lac*$^+$ equal to the overall proportion of *lac*$^-$ and *lac*$^+$ colonies found. Rather the h^+ mutants that gave rise to the *lac*$^-$ and *lac*$^+$ colonies appear to have arisen independently at different times during growth and to have multiplied into larger or smaller clones

TABLE 2

Variance analysis of the numbers of early- and late-arising h$^+$ *colonies on H$^-$ plates inoculated with the entire contents of replicate* h$^-$ *cultures*

	Experiment							
	1	2	3	4	5	6	7	8
Number of cultures	10	25	27	26	50	22	41	45
Bacteria per culture ($\times 10^7$)	3.2	2.2	3.4	3.6	3.8	3.2	38	24
Early-arising h^+ colonies								
Time in hours	55	49	52	51	55	47	48	53
Average number of h^+ colonies	3.3	6.3	28	1.2	17	5.4	52	66
Variance	31.2	642	2218	11.4	844	92.3	5009	701
P of homogeneity (χ^2 test)	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Late-arising h^+ colonies								
Time in hours*	142	192	218	191	170	214	192	101
Average number of h^+ colonies	0.8	0.5	1.3	0.7	1.2	1.5	3.3	5.5
Variance	0.9	0.7	2.1	0.8	1.4	1.4	8.1	9.9
P of homogeneity (χ^2 test)	0.22	0.14	0.02	0.68	0.37	0.46	<0.01	0.04

* Minus time at which early-arising colonies were enumerated.

accordingly. The late-arising colonies, on the other hand, did not show a clonal nature and their distribution fit (χ^2 test; $P = 0.29$) the hypothesis that they had a chance of being *lac*$^-$ or *lac*$^+$ according to the overall proportion found. Therefore, the late-arising h^+ colonies show no evidence of a clonal origin during growth. It can be concluded that the clones of h^+ mutants had their origin among the non-dividing h^- bacteria on the H$^-$ plate.

Clones of h^+ mutants also arise among h^- bacteria kept in the stationary phase in liquid. This was discovered in the course of using, for the determination of mutation rates, liquid cultures inoculated with ca. 50 h^- bacteria whose growth was limited by the exhaustion of histidine. If one or more mutations to h^+ occurred during the limited growth of the h^- bacteria, the h^+ mutants, unrestricted by the exhaustion of histidine, overgrew the culture and caused visible turbidity. From the proportion of cultures that did not show turbidity the average number of *mutations* can be calculated by the zero term of the Poisson formulation. It was observed that many cultures showed adaptive overgrowth at about 50 hours but that thereafter, at a low rate, new adaptive overgrowths occurred in other cultures for as long as 2 weeks. In order to determine how much time was required for the adaptive overgrowth of an h^+ mutant once it had appeared, single h^+ mutants which were also *lac*$^-$ were introduced into the unadapted h^- *lac*$^+$ cultures from a Poisson experiment. These mutants overgrew in about 27 hours. The time required for the h^- inoculum to grow to the level limited by the exhaustion of histidine (ca. 2.0×10^7 h^- bacteria per culture containing 0.25 μg L-histidine monohydrochloride) was between 20 and 24 hours. Since most of the cultures had no mutations, most of the

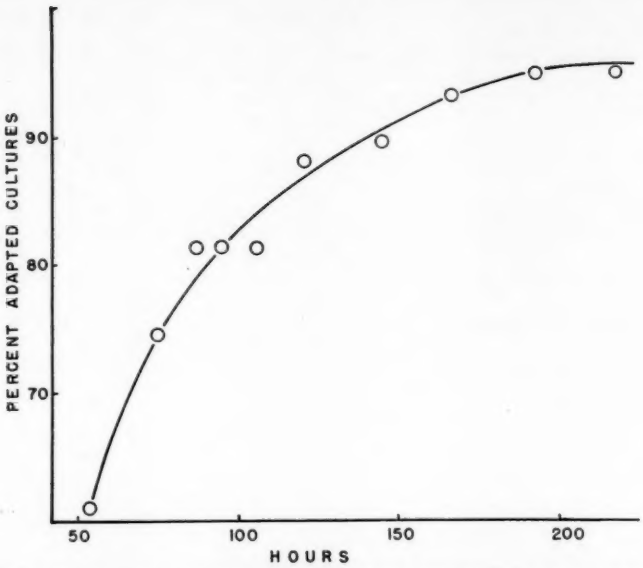


FIGURE 2.—The continued appearance of h^+ adaptations among h^- cultures in a histidine-limited stationary phase. The data are from 59 two ml cultures with 0.36 μg histidine which supported the growth of 3.2×10^7 h^- bacteria. The rate of mutation from h^- to h^+ during growth, calculated from the fraction of non-adapted cultures at 54 hours, was 2.0×10^{-8} per bacterium per generation.

mutants came from mutations which took place in the last generation. Therefore, the time at which an h^+ mutant arising during growth should be seen overgrowing the culture is about 50 hours.

But what about those mutants that overgrow some cultures after that time? Figure 2 shows the pattern of appearance of cultures with such mutants. The total number of h^- bacteria in these histidine-limited cultures remained absolutely constant when determined with a Petroff-Hauser counting chamber. For example, in 2 experiments, where the average total number of bacteria was 3.5×10^7 per tube after 1 day, that number was retained throughout the next 10 days at which time the average total number was 3.3×10^7. Hence, residual growth cannot have yielded the h^+ mutants that overgrew. Furthermore, the late adaptations involve growth of the h^+ mutants, from levels as low as 50 h^+ per culture, at a rate of ca. 56 minutes per generation. Since this is the maximal rate of growth of h^+ bacteria, the late adaptations cannot be said to be due to slow-growers. Like the late-arising h^+ colonies on H^- plates, the late adaptations appear to involve clones of h^+ mutants that arose among h^- bacteria in the stationary phase of the culture.

During the course of these long-term experiments attempts were made to retard evaporation; plates were stored at 37°C in a humid incubator; 2 ml liquid cultures were kept at 37°C in small tubes in a moist chamber. Nonetheless, some evaporation did occur. It did not reduce the volume of 2 ml cultures more than 10 percent

until after about 150 hours; later on it became more serious and calculations were corrected when it was observed to occur.

The rate of increase of the number of new h^+ mutant clones (m) due to mutation from h^- in the stationary phase can be written as:

$$\frac{dm}{dt} = \mu N \tag{1}$$

where μ is the chance of mutation per h^- bacterium per hour and N is the number of viable h^- bacteria present at time t which is measured in hours.

The rate of change of N because of mutation to h^+ alone can be expressed as:

$$\frac{dN}{dt} = -\mu N \tag{2}$$

and the rate of change of N because of death of the h^- bacteria alone (fig. 3) can be expressed as:

$$\frac{dN}{dt} = -kN \tag{3}$$

where k is the chance of death per hour. Thus the rate of change of N for these two reasons is:

$$\frac{dN}{dt} = -(\mu + k)N \tag{4}$$

The integral of equation 4 is:

$$N = N_o e^{-(\mu+k)t} \tag{5}$$

However, $\mu + k$ can be taken from the line in figure 3 as 1.8×10^{-2} and μ is less than 10^{-8}; therefore, $\mu + k$ is approximately equal to k and equation 5 can be approximated by:

$$N = N_o e^{-kt} \tag{6}$$

Equation 6 can be substituted into equation 1 to give

$$\frac{dm}{dt} = \mu N_o e^{-kt} \tag{7}$$

whose integral is:

$$m = N_o \frac{\mu}{k}(1 - e^{-kt}) \tag{8}$$

which transpsosed, give:

$$\mu = \frac{km}{N_o}(1 - e^{-kt}) \tag{9}$$

With equation 9 the rate of origin of new h^+ clones (mutation to h^+) can be calculated throughout the stationary phase.

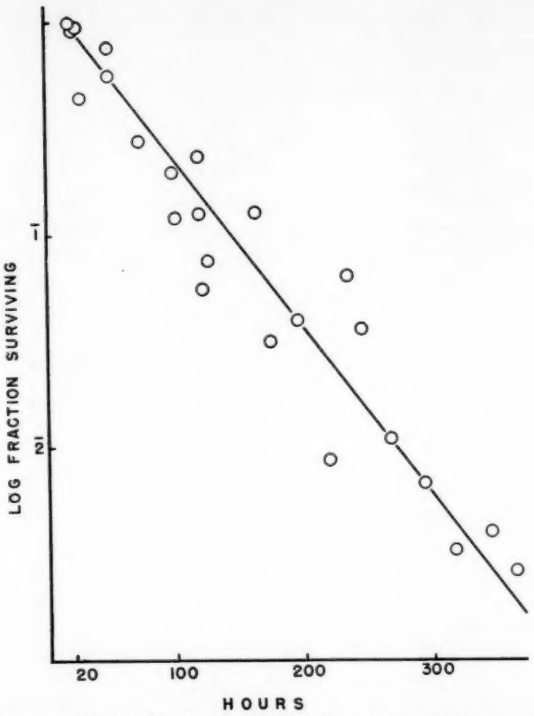


FIGURE 3.—The rate of death of h^- bacteria kept at 37°C in the stationary phase by the exhaustion of histidine.

The value N_o was calculated as follows. At about 50 hours an observation of the early-arising adaptations was made. At intervals thereafter the number of new adaptations was observed. Since 27 hours had to elapse between the origin of an h^+ mutant clone and its identification as an adaptive overgrowth, the adaptations observed during the interval had their mutational origin in the period between 27 hours before the beginning of the interval and 27 hours before its end. N_o was taken from figure 3 as the number of bacteria surviving at the time the interval began, minus 27 hours.

Table 3 shows, as an example, mutation rates calculated from the data in figure 2. It will be observed that the rate of appearance of new h^+ clones remains relatively constant throughout the stationary phase. This constancy was observed in eight other experiments, some of which involved stationary phases more than 300 hours long. The average rate of mutation in all these experiments was 1.2×10^{-9} per h^- bacterium per hour.

If it is assumed that death of h^- bacteria on H^- agar occurs at the same rate as in liquid, equation 9 can be used to calculate the rate of appearance of h^+ colonies

TABLE 3

Mutation rates from h^- *to* h^+ *in populations of* h^- *bacteria in a histidine-limited stationary phase in liquid medium*

Interval in hours	Average number of new mutations (m) from fraction of cultures without mutants	Chance of mutation per bacterium per hour (μ)
54–75	0.43	0.9×10^{-9}
75–87	0.31	1.6×10^{-9}
87–95	0	0
95–106	0	0
106–121	0.46	3.2×10^{-9}
121–145	0.15	0.9×10^{-9}
145–167	0.40	4.2×10^{-9}
167–193	0.30	3.9×10^{-9}
193–217	0	0
		average = 1.6×10^{-9}
54–193	2.05	1.5×10^{-9}

TABLE 4

Mutation rates from h^- *to* h^+ *in populations of non-dividing* h^- *bacteria on* H^- *plates*

Interval in hours	Average number of new h^+ colonies per plate (m)	Chance of mutation per bacterium per hour (μ)
47–68	0.56	1.6×10^{-9}
68–95	0.24	0.8×10^{-9}
95–122	0.16	0.9×10^{-9}
122–143	0.20	2.2×10^{-9}
143–166	0.24	3.6×10^{-9}
166–191	0.12	2.5×10^{-9}
191–215	0	0
		average = 1.7×10^{-9}
47–191	1.52	1.3×10^{-9}

among the h^- bacteria surviving on the plate. The early-arising colonies were counted after 2 days and new colonies were counted at intervals thereafter. N_o was taken from figure 3 as the number of bacteria surviving at the time at which the interval began, minus 4 hours. This was done because the colonies observed had an origin about 24 hours beforehand while death of the h^- parents on the plates began immediately and not after 20 hours of growth as was the case in liquid. Table 4 shows the mutation rates calculated from the data in figure 1. The relative constancy of the mutation rates observed is consistent with the assumption that h^- bacteria were dying on the plates. This constancy was found in six other experiments and the average mutation rate was 0.91×10^{-9} per h^- bacterium per hour.

This rate is indistinguishable from that found in liquid cultures and implies that the new h^+ colonies on H^- agar and the new adaptations in histidine-limited liquid cultures of h^- bacteria have the same origin.

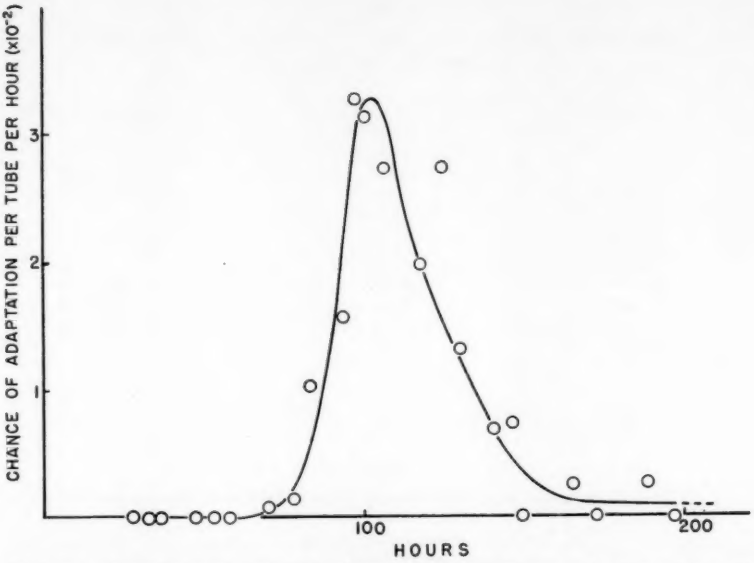


FIGURE 4.—The fraction of unadapted cultures adapting per hour as a function of time. The data are from an experiment in which 92 tubes containing 10 ml of H^- medium each received 0.1 ml of a suspension of h^- bacteria which had been subjected to a dose of ultraviolet light giving a survival of 1.1×10^{-5}. The frequency of induced h^+ mutants was 2.3×10^{-4} per survivor; the frequency of spontaneous h^+ mutants is ca. 2×10^{-7}.

It is now necessary to consider the possibility that the late-appearing clones of mutants are due to mutations which arose during the growth of the inoculum but which, even in the presence of histidine, ceased growing as soon as they arose but did not die. They would, consequently, not show a clonal distribution and would not be observed on H^- agar or in H^- medium until the onset of their growth which may not take place until well into the stationary phase of their h^- parents. This hypothesis in its simplest form would predict that there would be some average time at which the delay in the onset of division of the mutant would end. Thus the pattern of appearance of new mutant clones would be a distribution about some median. On the contrary, new mutant clones appear at a constant rate.

There is one instance known in which new mutants have a unique delay in the onset of division. This is the case of h^+ mutants induced by high doses of ultraviolet light (RYAN 1954). The pattern of termination of this delay was experimentally determined by tubing aliquots of a washed suspension of heavily irradiated h^- bacteria in H^- medium. If the dilution was so adjusted that each tube received on the average about one h^+ mutant, then the times at which they were observed to overgrow the cultures would very nearly reflect the times at which individual mutants begin to grow. Figure 4 shows how these times are distributed about a median. In this and similar experiments the h^- bacteria inoculated underwent some growth

from ca. 6×10^3 to ca. 5×10^5 per culture, presumably on histidine released from the dead cells present. Among the tubes still unadapted at the time this growth was observed to have occurred, one spontaneous mutation to h^+ could have occurred at the rate normal for growing h^- bacteria; an insignificant correction was accordingly made.

Most of the adaptations due to induced mutants occurred between 4 and 6 days; thereafter adaptations continued at the relatively low and constant rate of stationary phase mutation. At 6 days 26 percent of the tubes were unadapted, indicating that an average of 1.35 induced mutants had been added per tube. This is exactly the number of mutants calculated to have been added from a determination of the h^+ present in the irradiated h^- suspension by plating on H^- agar. In two other experiments, where the induced mutants added were also recovered as adaptations, a distribution of times of onset of growth around a median was found as in figure 4. The median time was shorter when survival was greater and the frequency of induced mutants lower; but the chance that an induced mutant would begin to grow was not constant with time. It can be concluded, therefore, that it is unlikely that spontaneous h^+ mutants, if they do have a long delay in the onset of growth, will have a constant chance to begin to divide with time. Hence this hypothesis for the appearance of new mutant clones in the stationary phase may be rejected.

DISCUSSION

There remain two hypotheses alternative to that of mutation in non-dividing bacteria which might still explain the data. The first is that genotypic changes occur during the growth of the inoculum in histidine but that the phenotypic change which allows the mutant to be recognized as h^+ occurs without division at some time during the stationary phase of the h^- parents (WRIGHT 1953b and c). WITKIN (DEMEREC *et al.* 1949) has interpreted the increased frequency of phage resistant mutants of *E. coli* during the "stationary" phase in broth when sodium nucleate is present as due to the acceleration of phenotypic expression by this compound. But, first, evidence has not been presented that this compound did not stimulate bacterial division and, second, the hypotheses that the nucleate hastened the delayed onset of division of mutants or raised the rate of mutation in the "stationary" phase were not excluded. Further, if nucleate simply accelerated phenotypic expression it would not be expected to produce zero-point mutants, a result reported to have occurred. It cannot be considered, then, that release from a phenotypic delay has been shown to occur in the stationary phase. With regard to the experiments reported in the present paper, this hypothesis, like that of the delayed onset of growth of the mutant, predicts a distribution of late-appearing mutants around a mean. This does not occur. Neither is there a clonal distribution of late-arising mutants which would ensue if the late-appearing mutants resulted from the delayed phenotypic expression of mutations occurring during growth of the inoculum in histidine. Therefore, phenotypic lag can be rejected as an explanation of the results.

The second hypothesis contends that bacteria in a histidine limited stationary phase are in a dynamic state and subject to turnover. Some cells may lyse and

provide histidine with which new cells are formed. In the course of this replacement by growth, mutations may occur at the rate normal for growing bacteria. Yet the total number of bacteria would remain the same, the growth being cryptic. It is possible to calculate what this rate of turnover must be if the mutations observed were actually occurring during growth at a rate of ca. 3×10^{-8} per bacterium per generation. Since there were on the average 2×10^{7} bacteria present per culture and mutations were occurring at a rate of ca. 1×10^{-9} per hour per bacterium, there were 2×10^{-2} mutations per hour per culture. When mutations occur at a rate of 3×10^{-8} per bacterium per generation, there are, on the average, 2×10^{7} bacteria per mutation. When the number of bacteria per mutation is multiplied by the number of mutations per hour, it is found that 4×10^{5} bacteria must divide per hour. This, divided by the number of bacteria per culture gives the turnover rate as 2×10^{-2}. Therefore, about one cell in fifty would have to lyse and be replaced each hour in order to account for the mutation rate observed in terms of mutation at the normal spontaneous rate during cryptic growth. This hypothesis demands not only an extraordinarily rapid spontaneous lysis for which there is no evidence, but also a remarkable efficiency of utilization of histidine released by lysis. Nonetheless, because it is amenable to experimental analysis it cannot be summarily rejected; a final decision on this point must wait until the completion of experiments now in progress.

In the meanwhile, the fact that mutations can arise in populations of bacteria whose numbers are not increasing must be accepted. In the present case the stationary phase was *bona fide* inasmuch as the total number of bacteria remained constant; thus, it is unlike the cases discussed by LWOFF and AUDUREAU (1941), ENGLESBERG and STAINER (1949) and RYAN (1952), where cryptic changes in population size were occurring. It may be similar to the case reported by KAPLAN (1947), where new mutants accumulated at a constant rate in colonies of *Serratia marcescens* in the stationary phase. Analogous cases appear to exist with organisms other than bacteria. For example, spontaneous mutations occur in spores of Streptomyces where the nuclei are not dividing (WAINWRIGHT unpublished), in the stored seeds and pollen of plants (STUBBE 1935, 1936; BLAKESLEE 1954) and in the stored sperm of Drosophila (TIMOFEEF-RESOVSKY 1937; MULLER 1946).

If these stationary phase mutations are actually occurring in the absence of a cryptic division, they occur as a constant function of physical time. This is the same relation as that found by NOVICK and SZILARD (1949) for mutations to phage resistance among bacteria dividing at different rates, although it is not yet certain how general this relation is (WITKIN 1953; DE ROTHSCHILD 1954). The magnitude of the mutation rate could be some function of the rate and nature of bacterial metabolism which is certainly lower and different during the stationary phase.

The reason mutation in the stationary phase of bacteria has not been more frequently observed is probably its low rate of occurrence. The simple observation of the total number of mutants before and after a short period in the stationary phase is not a sensitive enough method (RYAN and WAINWRIGHT 1954); there are too many mutants present after growth and too few added by stationary phase mutation to make the detection of the latter easy. Nor do stationary phase mutations

occur rapidly enough to increase the numbers of mutants in stock cultures significantly.

It is appropriate to point out here what constitutes a mutation like that from h^- to h^+ in asexually reproducing *E. coli*. What is observed is an hereditary change in cell type; from this is inferred a mutation in a genetic determinant. This is formally the same as any designation of genes as mutable units in sexually reproducing forms, such as strain K12 of *E. coli* or higher organisms. There are numerous and obvious resemblances between mutable genetic units in all organisms; the problem of the degree of similarity between mutable units and other gene concepts such as recombination units is one that cannot as yet be analyzed in the asexual h^- strain. As a consequence it is not known whether the mutable h^- genetic units are chromosomal or otherwise. It does appear, however, that they reside in the deoxyribonucleic acid "nuclear" bodies (RYAN, FRIED and SCHWARTZ 1954).

As a consequence, it is possible to imagine that an increase occurs in the genetic deoxyribonucleic acid in stationary phase cells that are not undergoing division. Thus, these stationary phase mutations could arise as errors in duplication. It is not, however, necessary to assume this, inasmuch as mutations are known to be induced by naturally occurring metabolites (cf. NOVICK and SZILARD 1952 and WRIGHT 1953). Although certain stages in the division cycle may be more sensitive, even non-dividing cells can respond to chemical mutagens. Natural radiation does not seem sufficient to account for rates of mutation as fast as those occurring among bacteria (cf. MULLER 1930).

SUMMARY

New clones of mutant h^+ bacteria appear during the stationary phase of parental h^- bacteria in both liquid and agar media. During this time there is no increase in the number of parents. The new h^+ clones are not the result of the slow growth of, or the development of phenotypes by, mutants which arose during growth. Nor can the new h^+ clones result from the delayed onset of growth of mutants arising during previous growth. The origin of the new h^+ clones occurs at a rate of ca. 1×10^{-9} per bacterium per hour which may be constant for many days. This rate is one fortieth of that which is observed during growth (ca. 4×10^{-8} per bacterium per hour).

ACKNOWLEDGMENT

This work was supported in part by an American Cancer Society grant recommended by the Committee on Growth and by research grants from the National Institutes of Health, U. S. Public Health Service and the Atomic Energy Commission. The author also wishes to thank DR. REINHARD KAPLAN for criticisms of the manuscript and LILLIAN WAINWRIGHT, PHYLLIS FRIED and MIRIAM SCHWARTZ for performing many of the experiments.

LITERATURE CITED

BLAKESLEE, A. F., 1954 The aging of seeds and mutation rates. Ann. N. Y. Acad. Sci. **57**: 488–490.

DEMEREC, M., B. WALLACE, E. M. WITKIN, and G. BERTANI, 1949 The gene. Carnegie Inst. Wash. Ybk. **48:** 154–166.

ENGLESBERG, E., and R. Y. STAINER, 1949 The relationship between growth and mutation in *Pseudomonas fluorescens*. J. Bacteriol. **58:** 171–180.

KAPLAN, R. W., 1947 Spontane Mutabilität bei *Bacterium prodigiosum*. Z. Naturforsch. **2b:** 308–312.

LURIA, S. E., and M. DELBRÜCK, 1943 Mutations of bacteria from virus sensitivity to virus resistance. Genetics **28:** 491–511.

LWOFF, A., and A. AUDUREAU, 1941 Sur une mutation de *Moraxella Lwoffi* apte a se développer dans les milieux a l'acide succinique. Ann. Inst. Pasteur **67:** 94–111.

MULLER, H. J., 1930 Radiation and genetics. Am. Naturalist **64:** 220–251.

1946 Physiological effects on "spontaneous" mutation rate in Drosophila. Genetics **31:** 225.

NEWCOMBE, H. B., 1953 The delayed appearance of radiation induced change in bacteria. Genetics **38:** 134–151.

NOVICK, A., and L. SZILARD, 1950 Experiments with the chemostat on spontaneous mutations of bacteria. Proc. Nat. Acad. Sci. U. S. **36:** 708–719.

1952 Anti-mutagens. Nature **170:** 926–927.

RYAN, F. J., 1952 Adaptation to use lactose in *Escherichia coli*. J. Gen. Microbiol. **7:** 69–88.

1954 The delayed appearance of mutants in bacterial cultures. Proc. Nat. Acad. Sci. U. S. **40:** 178–186.

RYAN, F. J., P. FRIED, and M. SCHWARTZ, 1954 Nuclear segregation and the growth of clones of bacterial mutants induced by ultraviolet light. J. Gen. Microbiol. **11:** 380–393.

RYAN, F. J., and L. K. SCHNEIDER, 1949 Mutations during the growth of biochemical mutants of *Escherichia coli*. Genetics **34:** 72–91.

RYAN, F. J., and L. K. WAINWRIGHT, 1954 Nuclear segregation and the growth of clones of spontaneous mutants of bacteria. J. Gen. Microbiol. **11:** 364–379.

STUBBE, H., 1935 Weitere Untersuchungen über Samenalter und Genmutabilität bei *Antirrhinum majus* L. Zeit. Ind. Abst. Ver. **70:** 533–537.

1936 Die Erhöhung der Genmutationsrate in alterndem Gonen von *Antirrhinum majus* L. Biol. Zentr. **56:** 562–567.

TIMOFEEF-ROSOVSKY, N. W., and K. G. ZIMMER, 1941 Über Zeitproportionalität und Temperaturabhängigkeit der spontanen Mutationsrate von Drosophila. Zeit. Ind. Abst. Ver. **79:** 530–537.

WITKIN, E. M., 1947 Mutations in *Escherichia coli* induced by chemical agents. Cold Spring Harbor Symposia Quant. Biol. **12:** 256–259.

1953 Effects of temperature on spontaneous and induced mutations in *Escherichia coli*. Proc. Nat. Acad. Sci. U. S. **39:** 427–433.

WRIGHT, B., 1953a The effect of α-hydroxy acids on reversion of a glycineless *E. coli* mutant. Compt. Rend. Trav. Lab. Carlsberg, Ser. Physiol. **25:** 173–211.

1953b Specific suppression by glycine of the phenotypic expression of partially reverted glycineless *E. coli* mutants. Nature **172:** 308–310.

1953c Auto-adaptation: a new phenomenon observed in a bacterial population. J. Bacteriol. **66:** 407–420.

DIRECT PROOF OF A VARIEGATED-TYPE POSITION EFFECT AT THE WHITE LOCUS IN DROSOPHILA MELANOGASTER[1]

BURKE H. JUDD[2]

Genetics Foundation, Department of Zoology, University of Texas, Austin, Texas

Received March 15, 1955

VARIEGATED-type position effect in *Drosophila melanogaster* has received a considerable amount of attention from many investigators (See review by LEWIS 1950). Direct proof of this phenomenon has been obtained for only two cases however. DUBININ and SIDOROV (1935) showed that the action of the gene for hairy (*h*) was affected when this locus was placed near heterochromatin of the fourth chromosome by a translocation between the third and fourth chromosomes. PANSHIN (1935) studying another 3-4 translocation showed that the curled gene (*cu*) also became unstable when placed near heterochromatin. By taking advantage of a small amount of crossing over between the variegating gene and the break point of the translocation, these workers were able to show in both cases that the gene in question behaved as the standard + allele when placed in a chromosome of normal structure. Proof of the position effect came from a comparison of translocation heterozygotes that differed in the position of the + and mutant genes. Variegation occurred in heterozygotes having the + allele in the rearranged chromosome and the mutant allele in the normal homolog, while heterozygotes in which the position of these two alleles had been exchanged gave a wild type phenotype. It was shown that the variegation is not the result of structural heterozygosis but depends only on the position of the + allele.

The present investigation provides direct proof of variegated-type position effect for the white locus. White-mottled types have been widely studied but only indirect evidence for position effect has been provided (SCHULTZ 1936; SACHAROV 1936; DEMEREC 1941; and others). The white gene provides more satisfactory material for working out this direct proof than does either curled or hairy. DUBININ and SIDOROV and particularly PANSHIN were dependent on the phenotype of a population of flies in order to determine the presence or absence of variegation. In the case of white, variegation, or the absence of it, can be detected easily in a single individual since there are some 800 facets in each eye and a change of color in only a few facets can easily be seen.

EXPERIMENTAL

The X-4 translocation $w^{258\text{-}21}$ (BRIDGES and BREHME 1944) was used for this study. The region to the left of 3E5 in the X chromosome has been moved to the heterochromatic region near the centromere of chromosome 4. The X chromosome genes

[1] This work is part of the thesis submitted to the faculty of the California Institute of Technology in partial fulfillment for the degree of Doctor of Philosophy.

[2] Presently, Fellow in Cancer Research of the American Cancer Society, Genetics Foundation, Department of Zoology, University of Texas, Austin, Texas.

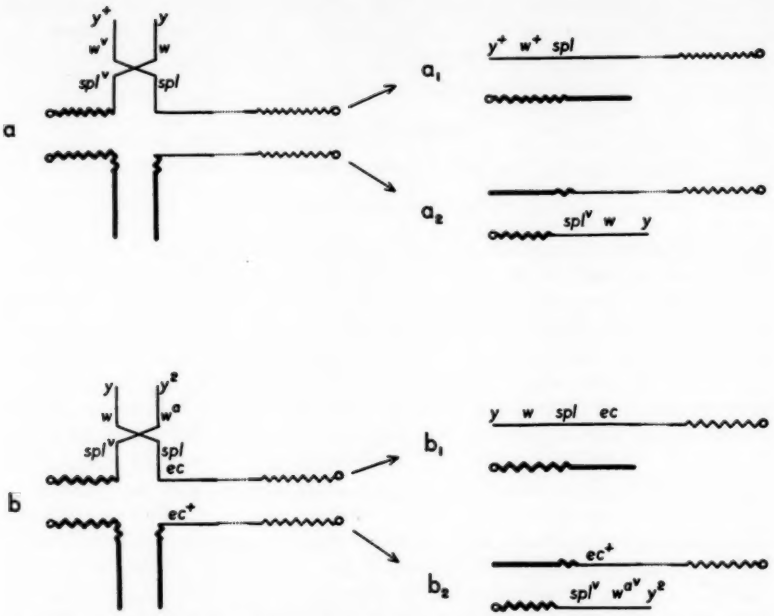


FIGURE 1.—Diagrammatic representation of $w^{258\text{-}21}$ heterozygotes, showing pairing relationships. The positions of the genes yellow (*y*), white (*w*), split (*spl*), and echinus (*ec*) are shown; variegation of a gene is indicated by the superscript *v*. The X chromosome is represented by the lighter lines while the heavier lines represent chromosome 4. Euchromatin is indicated by the straight lines and heterochromatin is shown by the zigzag lines. The centromeres are shown as open circles. A crossover between the white locus and the translocation break point is indicated and the resulting products are shown.

diminutive (*dm*), facet (*fa*), split (*spl*), and white (*w*) show variegation in flies heterozygous for the translocation and the mutant gene; echinus (*ec*) shows no variegation. This translocation also shows a variable Notch (*N*) phenotype and is lethal in the hemizygote.

The proof of position effect is based on the recovery and analysis of chromosomes in which a crossover has occurred between the white locus and the break point of the translocation. The basic experimental set-up is shown diagrammatically in figure 1. Females were obtained heterozygous for the translocation and a structurally normal X chromosome carrying the mutant genes yellow (*y*), white (*w*), split (*spl*), and singed-3 (sn^3) (fig. 1a). The second and third chromosomes were made heterozygous for chromosomal rearrangements since such an autosomal condition is known to be effective in increasing crossing over in the X chromosome (STEINBERG 1936). Two Curly (*Cy*) inversions (See BRIDGES and BREHME 1944) were used for the second chromosome. The left arm is marked by the dominant Curly (*Cy*), while the right arm carries speck-2 (sp^2). For the third chromosome the complex rearrangement Ubx^{130} (*Ubx* = Ultrabithorax, a dominant bithorax-like

change, LEWIS 1952) was used. These females were mated to *y w spl* sn^3 males and the offspring were examined for crossing over between *w* and *spl*. Two females of the phenotype *y w* spl^+ were found in approximately 22,000 female offspring examined. One of these females proved fertile, and gave *y w* and *y w spl* daughters and *y w spl* sons when mated to her *y w spl* sibs. A stock of the translocation now containing *y* and *w* (represented as product a_2 in fig. 1) was obtained by balancing with *In(1)sc*^{8}, *dl-49*, y^{31d} w^a lz^s (lozenge-spectacled) *B* (Bar). Females from the stock were crossed to *y* males and slides were made of the salivary glands from the *y* larvae. Cytological examination of the salivary gland chromosomes showed an X-4 translocation to be present with the breaks at the same points as described for the original $w^{258\text{-}21}$.

The complementary crossover class, diagrammed as product a_1 in figure 1, was also recovered. Two males of this class were found; both had normal wild type body and eye color (y^+ w^+). One carried *spl* while the other was spl^+; both were sn^3.

Since R(*w*)/w^+ flies prove to have a wild type eye color, as discussed below, it was felt that a more sensitive test for detecting the possibility of variegation in the heterozygote containing R(*w*) would be to employ a standard chromosome carrying an intermediate "allele" of white instead of w^+. The pseudoallele, (LEWIS 1952) apricot (w^a or *apr*), was chosen for this purpose. The genotypes to be compared for a precise position effect test were therefore R(*w*)/w^a and R(w^a)/*w*. For this reason the construction of R(w^a) was undertaken.

Part b of figure 1 shows the X chromosome constitution of the females used for this experiment. Females were obtained heterozygous for the translocation which carried *y w* and a normal chromosome having the mutant genes y^2 w^a *spl ec*; they were mated to males carrying *y w ec f* (forked). In the first of these matings the same autosomal rearrangements described above were used. No crossover products of the desired type were found in about 250 cultures examined and so other autosomal rearrangements were introduced. In one of the second chromosomes the rearrangement *SM1*, *al* (aristaless) *Cy* sp^2 was used and the homolog carried *In(2LR)102*, ds^w (dachsous-Wide) sp^2 (according to LEWIS and MISLOVE, personal communication, *SM1*, or "Second Multiple 1," refers to a complex of inversions consisting of a pericentric inversion with breakage points in 22A and 60B, superimposed on a chromosome carrying both left and right arm Curly inversions, while *In(2LR)102* has the following sequence: 21A to 26A/51D to 41/57A to 51D/26A to centromere of 2 to 41/57A to 60F). One of the third chromosomes contained Ubx^{130} and the other contained *In(3LP, 3RC) Sb* e^s (ebony-sooty). From this experiment two females with the phenotype yellow-2, dilute-apricot (w^a/*w*) were found among approximately 3,200 females examined. Both of these were the result of crossing over between *w* and *spl* and are represented in figure 1 as product b_2. Cytological examination of the salivary gland chromosomes confirmed the presence of the translocation.

The complementary crossover class was also recovered during these experiments. A total of five such crossovers were found, however, the total number of individuals examined was not recorded. Four of the crossovers occurred between *w* and *spl*; one took place between *spl* and the translocation break point.

A crossover between *ec* and the translocation break point would give the same phenotype (yellow-2, dilute-apricot) as the sought-after class; however, only three bands on the salivary gland chromosome map (BRIDGES 1938) separates these two points. Three females in which this type of crossover had occurred were picked up during these experiments. Males of this type would not be noticed since they would be y^2 w^a *spl* ec^+, thus indistinguishable for the most part from their y^2 w^a *spl ec* brothers.

To test the position effect in $w^{258\text{-}21}$, experiments were set up to compare the original translocation, R(w^+), with the newly substituted R(w). The variegation is known to be modified by the presence of an extra Y chromosome and by the temperature at which the culture is grown (GOWEN and GAY 1933, 1934). Therefore, single females from the R(w^+) and R(w) stocks were outcrossed to *In (1) dl-49, y Hw* (Hairy wing), m^2 (miniature-2), g^4 (garnet-4) males. Secondary non-disjunction in any of the females could be detected by this cross and in this way any XXY females could be eliminated. The R(w^+)/*In (1) dl-49, y Hw* m^2 g^4 offspring were mated to *y w ec f* or *y w spl* sn^3 males. The R(w)/*In (1) dl-49, y Hw* m^2 g^4 females were crossed with w^+ *spl* sn^3 or w^+ spl^+ sn^3 males, both types of which were described earlier as being derived by crossing over from the original translocation. One third of each of the two types of cultures were placed at 25°C, one third at 19°C, and the remaining one third were grown at 14°C. Each culture bottle was given a code number so that the genotype was not known until all the results had been tabulated. It was found that R(w^+)/w showed variegation for white; this was very marked in those cultures grown at 19°C and 14°C. The cultures of type R(w)/w^+ did not show mottling for white even when grown at these lower temperatures; although, as expected, both types of cultures showed variegation for split when the mutant *spl* was present in the chromosome of normal structure. All flies heterozygous for the translocation showed variegation for the dominant Notch; again this was more extreme in cultures grown at the two lower temperatures. To test the R(w) rearrangement further for the occurrence of variegation, females heterozygous for R(w) and each of the white alleles apricot (w^a), buff (w^{bf}), cherry (w^{ch}), coral (w^{co}), eosin (w^e), honey (w^h), ivory (w^i), satsuma ($w^{sa\,t}$), and tinged (w^t), were cultured at 19°C. No mottling was observed in the eyes of any of these females, while females heterozygous for these alleles and R(w^+) showed mottling in every case.

When the R(w^a) type was obtained, somewhat similar comparisons were made. R(w^a)/w gives a mottled pattern consisting of white or near-white spots on a dilute-apricot background, while R(w)/w^a gives a non-variegated dilute-apricot phenotype. R(w^a)/w^+ is wild type in phenotype with no indication of mottling. Variegation for split occurs in every case when *spl* is in the normal homolog.

DISCUSSION

The results from these experiments show conclusively that the somatic variegation in the expression of the white gene in the translocation $w^{258\text{-}21}$ is due to a position effect. The w^+ gene of the original translocation shows a variegated phenotype in the eyes of females heterozygous for the rearrangement and w. When this w^+

allele is transferred to an X chromosome of normal structure this variegation is lost and the gene functions as the dominant wild type allele. When the mutant gene *w* is placed in the translocation, no variegation is detected in flies of the type R(*w*)/w^+, even under low temperature culture conditions. When the intermediate pseudoallele of white, w^a, is placed in the translocation its action is affected such that R(w^a)/*w* flies give a mottled phenotype while R(*w*)/w^a flies show no trace of variegation.

The conclusion indicated by these facts is that a variability of action is conferred on the gene in the structurally abnormal chromosome while the allele in the structurally normal homolog retains its usual activity. The mutant allele *w* would not be expected to show any variability since the mutant gene itself is characterized by its loss of activity, that is, the same phenotype is exhibited by a deficiency for the white locus. The variegation in these cases must, therefore, be the result of the inactivation of the gene or its product when the gene in question is located in the rearranged chromosome.

This conclusion is not in agreement with the interpretation of the position effect phenomenon put forth by EPHRUSSI and SUTTON (1944) and supported by MULLER (1941, 1947). This is a structural hypothesis based on the assumption that the shape of the gene rather than its chemical composition may be altered in a way which is readily reversible. Variegation is interpreted as being the result of the upset of pairing relationships of the regions adjacent to the breaks of a chromosomal aberration in a heterozygous individual. A change in stress upon the gene might be expected to alter its activity. By this hypothesis, variegation should occur in R(*w*)/w^+ flies as well as the R(w^+)/*w* and R(w^a)/*w* types. The pairing relationships are expected to be precisely the same in these three genotypes, therefore, the variegation exhibited only by the two latter types cannot be due to structural heterozygosity alone. LEWIS (1950) has pointed out that the type of evidence presented here serves to dispose of the interhomolog influence in this type of position effect, which points up even more clearly the intrachromosomal position effect, in particular the euchromatin-heterochromatin association, as the fundamental problem to be considered.

SUMMARY

The variegation for white exhibited by the translocation $w^{258\text{-}21}$ is shown to be due to variegated-type position effect. The variegation is dependent on the variable effect of the allele placed in the proximity of heterochromatin in the structurally rearranged chromosome and is independent of the allele in the structurally normal homolog.

ACKNOWLEDGEMENTS

The author is deeply indebted to PROFESSORS E. B. LEWIS and A. H. STURTEVANT for their continued guidance and critical evaluation throughout this work.

LITERATURE CITED

BRIDGES, C. B., 1938 A revised map of the salivary gland chromosome of *Drosophila melanogaster*. J. Heredity **29**: 11–13.

BRIDGES, C. B., and K. S. BREHME, 1944 The mutants of *Drosophila melanogaster*. Carnegie Inst. Wash. Publ. No. **552,** vii + 257 pp.

DEMEREC, M., 1941 The nature of changes in the white-Notch region of the X chromosome of *Drosophila melanogaster*. Proc. 7th Inter. Congr. Genet. 99–103.

DUBININ, N. P., and B. N. SIDOROV, 1935 The position effect of the hairy gene. Biol. Zhur. **4:** 555–568.

EPHRUSSI, B., and E. SUTTON, 1944 A reconsideration of the mechanism of position effect. Proc. Nat. Acad. Sci. U. S. **30:** 183–197.

GOWEN, J. W., and E. H. GAY, 1933 Eversporting as a function of the Y-chromosome in *Drosophila melanogaster*. Proc. Nat. Acad. Sci. U. S. **19:** 122–126.

1934 Chromosome constitution and behavior in eversporting and mottling in *Drosophila melanogaster*. Genetics **19:** 189–208.

LEWIS, E. B., 1950 The phenomenon of position effect. Advances in Genet. **3:** 73–115.

1952 The pseudoallelism of white and apricot in *Drosophila melanogaster*. Proc. Nat. Acad. Sci. U. S. **38:** 953–961.

MULLER, H. J., 1941 Induced mutations in Drosophila. Cold Spring Harbor Symposia Quant. Biol. **9:** 151–165.

1947 The gene. Proc. Roy. Soc. London, B **134:** 1–37.

PANSHIN, I. B., 1935 New evidence for the position effect hypothesis. Compt. Rend. Acad. Sci. U.R.S.S. N. S. **4:** 85–88.

SACHAROV, V. V., 1936 The character mottled (w^{m^1}) in Drosophila as a result of the "position effect." Biol. Zhur. **5:** 293–302.

SCHULTZ, J., 1936 Variegation in Drosophila and the inert chromosome regions. Proc. Nat. Acad. Sci. U. S. **22:** 27–33.

STEINBERG, A. G., 1936 The effect of autosomal inversions on crossing over in the X chromosome of *Drosophila melanogaster*. Genetics **21:** 615–624.

THE GENETICS OF VIVIPARY IN MAIZE[1]

DONALD S. ROBERTSON

California Institute of Technology, Pasadena, California and Biola Bible College, 558 South Hope Street, Los Angeles 17, California

Received March 21, 1955

PREMATURE germinating seeds have been observed in various plants, including lemons, oranges, melons, wheat and rye. However, in none of these is there any good indication that the premature germination is the result of changes in the germ plasm. Only in maize has the genetic control of this phenomenon been clearly demonstrated. Such mutants have been described by MANGELSDORF (1923, 1926, and 1930), LINDSTROM (1923), EYSTER (1924a, 1924b and 1931b) and SPRAGUE (1936). These mutants are characterized by seeds that germinate while still attached to the ear, before full maturity has been reached. Usually the germination involves only the plumule with little or no germination of the radicle. Only in an occasional seed will both the root and plumule germinate prematurely.

Early reports of vivipary in maize by LINDSTROM (1923), MANGELSDORF (1923) and EYSTER (1924a and 1924b) were mainly descriptive. In later papers MANGELSDORF (1926 and 1930) described intercrosses of several viviparous mutants and was able to demonstrate a total of six different loci responsible for premature germination. In addition he postulated nine loci to explain aberrant ratios. EYSTER (1931a and 1931b) presented linkage data for four viviparous mutants: some of which will be considered below.

SOURCE OF GENES

This study includes the following mutants: *vp*$_1$, *vp*$_2$, *vp*$_4$, *vp*$_5$, *vp*$_6$, *vp*$_8$, *vp*$_9$, *ps* and *w*$_3$. Viviparous-1, *vp*$_2$ and *vp*$_4$ were obtained from the Maize Genetic Cooperation. Viviparous-5 which was supplied by DR. BURNHAM proved to be allelic to a viviparous mutant furnished by DR. SPRAGUE and to several mutants that appeared in the progeny of seeds exposed to X-ray and atomic irradiations. Viviparous-6 first appeared in stocks derived from seeds exposed at the Bikini atom bomb test. Whether the mutation was induced by the irradiation or present in the exposed seed could not be determined. The *vp*$_8$ mutant originally appeared in stocks derived from seed exposed at the Bikini atom bomb test. Viviparous-9 originated in the progeny of a seed exposed to an X-ray dosage of 20,000r units. Stocks of *ps* were supplied by DR. SPRAGUE. At least ten alleles of this mutant from different sources have been found. Stocks of the *w*$_3$ mutant were furnished by DR. PHINNEY.

DESCRIPTION OF THE MUTANTS

Besides inducing premature germination all the viviparous mutants so far tested have markedly affected the phenotype of the seed and/or seedling in other ways. By

[1] Portions of the work reported in this paper are included in a thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Division of Biology, California Institute of Technology. The remainder of the work herein reported was accomplished during post-doctoral fellowships from the same school for the summers of 1951, 1952, and 1953.

using these pleiotropic effects, two distinct classes of viviparous mutants can be recognized: Class One, in which the mutant gene does not have an effect upon the endosperm and seedling color; and Class Two, in which the mutant gene in addition to inducing premature germination also alters the carotenoid and chlorophyll synthesis. The mutants of the latter class have white or pale endosperms and albino seedlings.

Mutants of Class One

Viviparous-1

Allele tests between the stocks of vp_1 and vp_4 obtained from the Maize Genetic Cooperation indicate that these two mutants are allelic and the symbol vp_1 will henceforth be used for both of them. This mutant germinates in the late dough stage producing a green seedling that grows into a normal appearing mature plant. Seeds homozygous for this gene and carrying the genes required for aleurone color, A_1, A_2, C, R and i fail to develop color in this layer. Such seeds are entirely colorless or may develop a slight tinge of color, but in all cases the color is distinctly reduced compared to that of the non-viviparous seeds.

Viviparous-6

This mutant differs from vp_1 in that all viviparous seedlings so far grown have been necrotic. In this regard they resemble the ge_2 mutant described by MANGELSDORF (1926). Since there is no known source of the ge_2 mutant in existence today, it has been impossible to obtain an allelic test. This mutant does not interfere with the expression of aleurone color.

Viviparous-8

The expression of vivipary by this mutant is very variable. In some ears only one or two premature germinating seeds are observed. In no selfed ear has a clear cut 3:1 ratio been found. The principal effect of this gene on the seed seems to be in the region of the germ which in a mature seed is very shrunken. In such seeds it appears as if the endosperm adjacent to the embryo had been digested causing the seed to collapse in this region. All viviparous-8 seeds which have been studied show these characteristics in addition to germinating prematurely. The viviparous seeds produce weak, dwarf seedlings which have short internodes and narrow, short, pointed leaves. The mature plants are very dwarfed varying from about one to three feet in height. Most of the mature vp_8 plants produce functional tassels but only a few are strong enough to produce an ear. When ears are produced, they are very small and bear small seeds. This mutant does not interfere with the formation of color in the aleurone.

Mutants of Class Two

The inhibition of carotenoids and chlorophyll in the mutants of this class has been interpreted differently by EYSTER (1924a, 1924b, 1931b) and MANGELSDORF (1923, 1926). EYSTER suggested that a gene closely linked to vivipary was responsible for the lack of carotenoids and chlorophyll while MANGELSDORF proposed that the early germination of the viviparous seeds interfered with the normal formation of these

pigments. Both of these explanations were based on the observation of unexpected seeds that appeared on ears segregating for vivipary. These seeds had white endosperms and dormant embryos which produced green seedlings on germination. Since these seeds as well as the reciprocal class (yellow endosperm-albino viviparous seedling), which is occasionally observed, can be explained by heterofertilization (SPRAGUE 1929, 1932), the simplest explanation is that the viviparous gene is directly responsible for the lack of carotenoids and chlorophyll.

All the mutants of this class under conditions of low light intensities have been observed to produce a faint blue-green pigment which disappears when exposed to light. KOSKI and SMITH (1951) found this pigment in w_3 mutants grown in the dark to be protochlorophyll which is converted to chlorophyll-*a* when exposed to light for a short period of time and BANDURSKI (unpublished) found this pigment in vp_5 to be chlorophyll-*a* when analysed in the light.

Viviparous-2

The endosperm color of this mutant varies from a pale yellow to white and the seedlings produced are pure white if grown in the light. Sometimes these pale seeds fail to germinate prematurely. However, when they are germinated they always produce the white seedlings typical of this mutant. The exact reason why these seeds should fail to germinate prematurely is not known. Frequently such seeds are common on ears that have only a few loosely fitting husks which suggests that the maturing seeds might dry too fast to permit the premature germination.

Seeds homozygous for this mutant frequently are smaller than the normal seeds on the same ear. EYSTER (1931a) suggests that the reduction in size is caused by a closely linked gene. However, a mutant allelic to vp_2 which also affected the seed size was found by DR. ANDERSON in a stock derived from seed exposed to the Bikini atom bomb. The origin of vp_2 associated with small seed size at two different times would suggest that the vp_2 gene itself is responsible for the reduced size since it is unlikely that a mutation affecting this trait would be induced simultaneously with vp_2 on two different occasions.

Viviparous-5

The white or pale yellow seeds of this mutant give rise to white seedlings when grown in the light. BANDURSKI (unpublished) measured the total carotenoid content of vp_5 seedlings using a Beckman Model D. U. spectrophotometer at 440 μ and found no suggestion of a compound possessing typical carotenoid absorption. As in the case of vp_2, occasional dormant seeds with white endosperms which produce albino seedlings are observed on ears segregating for vp_5. But unlike vp_2, the vp_5 gene does not seem to reduce the size of the mutant seeds.

Viviparous-9

Phenotypically this mutant is very similar to vp_5. As a general rule vp_9 seeds germinate a little more vigorously than those of vp_5 and only very rarely is a dormant vp_9 seed observed.

Pink scutellum

This mutant was described by SPRAGUE (1936, 1945). It is similar to others of this class in that the seeds have an altered carotenoid synthesis and the viviparous seedlings are albino if grown in the light. However, they differ in that the endosperm of the mutant seeds instead of being white or pale yellow, is pink. The albino seedlings also are tinged with pink. Preliminary chromatographic tests of the carotenoid content of *ps* seeds revealed that it is strikingly different from that of normal seeds.

White-3

The symbol w_3 was first given to an albino seedling mutant by DEMEREC (1923). The same symbol was used by LINDSTROM (1924) for a similar mutant. Both authors suggested the possibility of their gene being identical to that of the other but no tests were made to establish this. In any case, neither worker mentioned that their w_3 gene interfered with developmental dormancy or carotenoid developments in the endosperm.

The original stocks of w_3 used in this study were homozygous white endosperm (y_1) which occasionally segregated viviparous seeds. When these stocks were crossed with standard yellow dent lines it became obvious that this was a class two viviparous mutant. The F_2 ears of such crosses were segregating for white seeds in a 9:7 ratio with only the white seeds being viviparous or giving rise to albino seedlings. On further backcrossing to standard stocks a line was isolated that segregated in a 3:1 ratio for white (or pale yellow) seeds that were viviparous and gave rise to albino seedlings.

SPECIAL TECHNIQUES USED IN PLACING GENES

Besides the conventional methods of locating genes by obtaining indications of linkage with known genes, two special techniques were used involving: 1) translocations between different chromosomes of the basic set and 2) translocations involving a chromosome of the basic set and a B-type chromosome.

After the gene was placed as to chromosome a modified test-cross was used to locate the gene on the linkage map. This was necessary because most of the viviparous plants do not survive beyond the seedling stage making it impossible to obtain homozygous viviparous plants for test-crossing.

Translocations

ANDERSON (1952) has described how translocations can be employed to make efficient use of the few excellent marker genes that affect the endosperm of maize. If translocations are selected which have breaks close to the loci of genes such as *su*, *y*, or *wx* while the other breaks are in various of the other chromosomes, these genes can be used to test for linkage in the chromosome not carrying the marker gene. In this way regions of the chromosomal complement that are lacking good marker genes can be tested. This is possible because the only functional pollen grains produced by plants heterozygous for a translocation are those which carry two translocated chromosomes and those which carry the two normal chromosomes. The pollen grains carrying the two translocated chromosomes will always include the two break points

and thus these points are completely linked. Similarly the corresponding points on the non-translocated chromosomes must be completely linked. Therefore, genes in one chromosome closely linked to the point of breakage will be linked to genes in the other chromosome close to the break point. If the gene in one of these chromosomes is a marker and the gene in the other is unplaced, effective linkage can be obtained even though they are not on the same chromosome. If the unknown gene is not linked to the marker gene in the absence of the translocation then linkage between the two genes in its presence means that the unplaced gene must be on the chromosome of the translocation not carrying the marker.

Translocations involving B-type chromosomes

The use of interchanges involving B-type chromosomes to locate unplaced genes has been previously outlined by ROMAN and ULLSTRUP (1951). This type of translocation is very effective since it makes possible the placing of unknown genes in the F_1 generation. Because of nondisjunction of the B-type centromere in the division of the generative nucleus, a given pollen grain will possess gametes that differ in constitution with respect to the portion of the chromosome attached to the B centromere. One of the gametes will have this portion present in duplicate while the other will be deficient for this region (ROMAN 1947). When such pollen functions in fertilization, seeds result in which the endosperm or embryo is deficient for that portion of the chromosome attached to the B centromere. Therefore, any recessive gene found in this region of the maternal chromosomes will be observed in the F_1 generation.

Modified testcrosses

In this type of testcross the F_1 plants were crossed to stocks that possessed the normal alleles for vivipary and were in most cases recessive for the genes with which linkage was desired. The testcross seeds and/or plants are then classified for characters with which linkage was being tested and the plants from such seeds self-pollinated to determine if they carried vivipary. Because this type of testcross requires the growing of mature plants in order to test vivipary the number of individuals that can be tested is relatively small. However, in spite of the small numbers, more reliable results can be expected than those obtained from F_2 data.

GENETIC STUDIES

Viviparous-1

EYSTER (1931b) reported that *vp*$_1$ was linked to *R* with only 0.15% crossing over and to *g* with about 35% crossing over. Viviparous-4, which in the present studies proved to be allelic to *vp*$_1$, was reported by EYSTER (1931b) to be linked to *sh* with 11.8% crossing over. Attempts to confirm these linkage relationships were not successful. The action of the *vp*$_1$ gene in inhibiting pigment formation in the aleurone would explain the apparent linkage of this gene to the *R* locus.

A plant heterozygous for *vp*$_1$ was pollinated by *TB-3a*. Viviparous seeds were observed on the F_1 ear. This would place the gene in the distal .9 of the long arm of three since this portion of three is attached to the B centromere.

TABLE 1

Expected genotypes when TB-3a *pollen functions on* vp_1 *silks*

Gametic unions	Genotype of endosperm	Genotype of embryo
Class A Hyperploid sperm—polar nuclei Deficient sperm—egg nucleus	*VP VP vp vp*	*— vp*
Class B Hyperploid sperm—egg nucleus Deficient sperm—polar nuclei	*— vp vp*	*Vp Vp vp*

Although all the seeds of this cross carried the genes for aleurone color, A_1, A_2, *C* and *R*, some had colorless aleurones. Three types of seeds could be distinguished with respect to aleurone color and vivipary: 1) colored aleurone, viviparous: 2) colorless aleurone, dormant; and 3) colored aleurone, dormant. If vp_1 seeds have colorless aleurones because the act of premature germination interferes with pigment formation in the aleurone, only the viviparous seeds on the above ear would be colorless while the nonviviparous seeds would be colored. Since this is not observed the vp_1 gene must act directly to inhibit pigment formation in this tissue.

The expected viviparous genotypes of seeds resulting from the union of *TB-3a* pollen which carries duplicate and deficient gametes with ovules that carry the vp_1 gene are shown in table 1. Since vp_1 acts directly on the aleurone in inhibiting color, the colored aleurone, viviparous seeds described above must belong to Class A while the colorless aleurone, dormant seeds must belong to Class B. The third type of seeds described above either do not carry the vp_1 gene or were fertilized by sperms from pollen grains in which nondisjunction did not take place.

If the above explanation is correct then a further conclusion can be drawn from this cross. SPRAGUE (1936) and ROBERTSON (1951) have presented evidence that in two other viviparous mutants (*ps* and vp_6 respectively) premature germination is determined by the genotype of the embryo and is independent of the genotype of the endosperm. This would also appear to be true for vp_1 since Class A seeds, which have genetically viviparous embryos and non-viviparous endosperms, germinate prematurely while those of Class B do not.

Viviparous-1 has also been placed with respect to *wx T3-9a*, *wx T3-9c* and a_1. Testcross data involving the translocations and a_1 are given in tables 2, 3 and 4. The *wx* — vp_1 distance is 13.7% for *wx T3-9a* and 11.1% for *wx T3-9c*. ANDERSON (1938) reports 3.6% crossing over between *T3-9a* and *wx* and 7.6% crossing over between *T3-9c* and *wx*. The difference between the *T-wx* distances and the *wx*-vp_1 distances should approximate the crossover values expected between the translocations and vp_1. This value is 10.1% for *T3-9a* and 3.5% for *T3-9c*. The linkage data with a_1 suggests very little linkage if any. The value of 48.0% total crossing over is close to that given by ANDERSON and BRINK (1940) of 45.1% for the recombination between *T3-9a* and a_1.

LONGLEY (unpublished) has placed the break point of *T3-9a* in the long arm of chromosome 3 about .19 of the distance from the centromere. ANDERSON (1938) has

TABLE 2

Linkage data between wx *and* vp_1 *from testcrosses involving* T3-9a, wx *and* vp_1

F_1 Parent	Parental combinations		Recombinations		Totals	Percent recombinations
	+ *vp*	*wx* +	++	*wx vp*		
729-2	122	121	29	24	296	17.9
729-2a	159	181	32	10	382	11.0
729–3	134	117	26	13	290	13.4
729-3t	58	62	12	10	142	15.5
729-4	131	131	21	7	290	9.7
729-5	63	64	19	6	152	16.4
729-6	151	140	33	18	342	14.9
729-7	66	74	15	6	161	13.0
729-9	86	99	16	13	214	13.6
730-4	87	64	11	7	169	10.7
730-9	99	92	28	9	228	16.2
730-9a	75	53	14	8	150	14.7
Totals	1231	1198	256	131	2816	13.7

TABLE 3

Linkage data between wx *and* vp_1 *from test crosses involving* T3-9c, wx *and* vp_1

F_1 Parent	Parental combinations		Recombinations		Totals	Percent recombinations
	+ *vp*	*wx* +	++	*wx vp*		
731-4	75	69	14	4	162	11.1
731-5	65	59	10	7	141	12.1
731-6	172	146	24	11	353	9.9
731-7	146	145	17	15	323	9.9
731-7a	93	79	19	6	197	12.7
731-8	61	74	13	4	152	11.2
731-9t	42	32	4	2	80	7.5
731-10	109	116	12	7	244	7.8
732-1	75	67	17	5	164	13.4
732-1t	54	65	8	6	133	10.5
732-2	99	95	20	14	228	14.9
732-7	103	80	13	8	204	10.3
732-8	93	79	19	6	197	12.6
Totals	1187	1106	190	95	2578	11.1

located the break point of *T3-9c* at about .1 of the way out on the long arm of three while LONGLEY (unpublished) has placed it at about .15. The positions of the break points in chromosome 3 of these two translocations is very close to that given by ROMAN and ULLSTRUP (1951) for the break of *TB-3a* which is located .1 of the way out on the long arm. Although the exact order is difficult to determine the cytological evidences and the crossing over data obtained in this study and that of ANDERSON and BRINK (1940) would suggest that vp_1 is proximal to the break point of *T3-9a*

TABLE 4

Test cross data involving A_1 *and* vp_1

F_1 Parent	Parental combinations		Recombinations		Totals	Percent recombinations
	A vp	++	*A* +	+ *vp*		
52-756-14	52	53	43	41	189	44.4
52-756-18	30	35	40	33	138	52.9
Totals	82	88	83	74	327	48.0

TABLE 5

Test cross data involving pr *and* vp_2

F_1 Parent	Parental combinations		Recombinations		Totals	Percent recombinations
	+ *vp*	*pr* +	++	*pr vp*		
50-1514-4	18	25	12	6	61	29.5
50-1514-7	40	19	8	6	73	19.2
50-1515-3	39	39	30	21	129	39.5
51-2752-7*	74	59	16	12	161	17.4
51-2738-41*	68	54	19	19	160	23.8
Totals	239	196	85	64	584	25.5

* Data from three point tests involving bm_1.

TABLE 6

Test cross progenies of $\frac{+\ bm_1\ pr}{+\ bm_1\ pr} \times \frac{vp_2\ +\ +}{+\ bm_1\ pr}$

F_1 Parent	Parental combinations		Recombinations						Totals	Percent recombinations	
			Region 1		Region 2		Region 1 & 2			Region 1	Region 2
51-2752-7	74	59	1	2	11	14	0	0	161	1.9	15.5
51-2738-41	68	51	0	4	19	15	0	3	160	4.4	23.1
Totals	142	110	1	6	30	29	0	3	321	3.1	19.3

about 10 units and distal to that of *T3-9c* about 3 units. This would place it about three units to the right of the *TB-3a* break point which is near that of *T3-9c*.

Viviparous-2

The results of previous studies made by EYSTER (1931a, 1931b) and BURNHAM (1935) had placed vp_2 on chromosome five. The linkages obtained in these studies were all based on F_2 data. In an attempt to obtain more reliable data testcrosses were made involving the genes *pr*, bm_1 and translocations *T5-9c*, *T4-5i* and *T4-5d*. The results of these tests are shown in tables 5, 6, 7, 8, and 9. Since bm_1 has been placed in the short arm of chromosome 5 by RHOADES (1936) and MCCLINTOCK

TABLE 7

Test cross progenies of $\frac{wx\ +\ +}{wx\ +\ +} \times \frac{wx\ T5-9c\ +}{+\ +\ vp_2}$

F_1 Parent	Parental combinations		Recombinations			Totals	Percent recombinations	
			Region 1	Region 2	Region 1 & 2		Region 1	Region 2
50-1511-1	70	74	2 0	0 0	0 0	146	1.4	0.0
50-1511-3	37	57	2 1	0 2	0 0	99	3.0	2.0
50-1511-4	64	63	2 5	0 1	1 0	136	5.9	1.5
Totals	171	194	6 6	0 3	1 0	381	3.4	1.0

TABLE 8

Test cross progenies of $\frac{+\ +}{+\ +} \times \frac{T4\ -\ 5i\ +}{+\ vp_2}$

F_1 Parent	Parental Combinations		Recombinations		Totals	Percent recombinations
	$+\ vp$	$T\ +$	$++$	$T\ vp$		
50-1512-30	61	68	1	2	132	2.3
50-1512-16	67	82	0	0	149	0.0
Totals	128	150	1	2	281	1.1

TABLE 9

Test cross progenies of $\frac{su\ +\ +}{su\ +\ +} \times \frac{su\ T\ 4\ -\ 5d\ +}{+\ +\ vp_2}$

F_1 Parent	Parental combinations		Recombinations			Totals	Percent recombinations	
			Region 1	Region 2	Region 1 & 2		Region 1	Region 2
52-790-5	40	66	2 0	2 2	0 0	112	1.8	3.6

(1938, 1941), the linkage data with *pr* and *bm*, would indicate that vp_2 is also in the short arm about 3.1 units to the left of bm_1 and 25.5 units from *pr* which is in the long arm. The position of vp_2 with respect to *T5-9c* and *T4-5i* is more difficult to determine. LONGLEY (unpublished) has placed the break points of these chromosomes at S.21 and S.13 respectively. Since both of these translocations showed about 1% crossing over with vp_2 it is possible that the gene is located between them. Further tests will be needed before this gene can be placed exactly with respect to these translocations. The translocation *T4-5d*, which LONGLEY (unpublished) has placed in the long arm of five at L. 19, shows about 3.6% crossing over with vp_2.

Viviparous-5

Plants heterozygous for vp_5 pollinated by *TB-1b* pollen produce ears segregating for vivipary. Since the distal 95% of the short arm of Chromosome 1 is transferred

TABLE 10

Test cross progeny of $\frac{+\ +\ +}{+\ +\ +} \times \frac{\text{wx T1} - \text{9a} +}{+\ +\ \text{vp}_5}$

F_1 Parent	Parental combinations	Recombinations			Totals	Percent recombinations	
		Region 1	Region 2	Region 1 & 2		Region 1	Region 2
49-5839-3	32 38	5 0	17 14	0 1	107	5.6	29.9

TABLE 11

Test cross progeny of $\frac{+\ +\ +}{+\ +\ +} \times \frac{+\ +\ \text{vp}_5}{\text{wx T1} - \text{9c} +}$

F_1 Parent	Parental combinations	Recombinations			Totals	Percent recombinations	
		Region 1	Region 2	Region 1 & 2		Region 1	Region 2
51-2750-5	63 80	4 2	19 31	2 0	201	4.0	25.9

TABLE 12

Test cross progeny of $\frac{+\ +}{+\ +} \times \frac{\text{T1} - \text{3a} +}{+\ \text{vp}_5}$

F_1 Parent	Parental combinations		Recombinations		Total	Percent recombination
	T +	+ *vp*	*T vp*	++		
50-1681-5	45	40	24	20	129	34.1

to the B centromere in this translocation vp_5 must be somewhere in this region. Modified testcrosses involving vp_5 and translocations *T1-9a*, *T1-3a* with and without *P*, and *T1-9c* were made in order to place this gene more accurately. The results of these crosses are seen in tables 10, 11, 12, and 13. The crossover values given in table 13 should be disregarded since the cross involving F_1 Parent 52-794-4 did not give the expected 1:1 ratio for *T* and vp_5 and since the numbers involved in the second cross are too small to be very dependable. However, the data are useful in determining the relative positions of the genes and translocation upon the chromosome.

LONGLEY (unpublished) has placed the breaks of these translocations in chromosome one as follows: *T1-9a*, S.17; *T1-9c*, S.54; and *T1-3a*, S.26. The cytological and crossing over data can be utilized to approximate the position of vp_5 on chromosome one although further tests will be needed to determine the cause of the discrepancy in cytological and linkage data for *T1-3a* and *T1-9a*.

ANDERSON (1941) in studies including these translocation found that their break points are all to the right of *P*. The linkage data from table 13 indicate a sequence $vp_5 - P - T1\text{-}3a$. Thus vp_5 must be to the left of *P* about 25.9 units from *T1-9c*, 29.9 units from *T1-9a* and 34.1 units from *T1-3a*. The data will not permit a reliable estimate of the $vp_5 - P$ distance.

TABLE 13

Test cross progeny of $\frac{+\ +\ +}{+\ +\ +} \times \frac{+\ \text{P}\ \text{T1}\ -\ 3\text{a}}{\text{vp}_8\ +\ +}$

F_1 Parent	Parental combinations	Recombinations						Totals	Percent recombinations	
		Region 1		Region 2		Region 1 & 2			Region 1	Region 2
52-794-4	38	77	34	13	13	7	2 2	186	27.4	12.9
52-794-1	11	14	9	11	4	6	2 0	57	38.6	21.1

TABLE 14

Test cross progenies involving T1-7c, Br, vp_8 *and* bm_2

F_1 Parent	Genotype of F_1 parent	Parental combinations		Recombinations				Region 1*		Region 2		Region 1 & 2		Totals	Recombinations	
				Region 1*		Region 2									Region 1	Region 2
50-1543-9	$\frac{T\ +\ bm}{+\ vp\ +}$	27	28	23	13	4	6	1	0	102	36.3	10.8				
50-1543-21a	$\frac{T\ +\ bm}{+\ vp\ +}$	11	17	8	5	3	0	0	1	45	31.1	8.9				
50-1543-21b	$\frac{T\ +\ bm}{+\ vp\ +}$	30	26	16	14	3	2	1	1	93	34.4	7.5				
50-1543-26	$\frac{T\ br\ +}{+\ +\ vp}$	7	8	1	6	—	—	—	—	22	31.8					
50-1543-29	$\frac{T\ br\ +\ bm}{+\ +\ vp\ +}$	9	10	4	8	5	1	0	1	38	34.2	18.4				
Total Region 1				98				5		300	34.3					
Total Region 2						24		5		278		10.4				

* A total of 175 plants were classified for the translocation and *br* without obtaining a crossover between them; therefore, in this summary the regions *T-vp* and *Tbr-vp* are both considered region 1.

Viviparous-8

A plant heterozygous for vp_8 pollinated by pollen from a *TB-1a* plant produced an ear segregating viviparous seeds. Pollination using *TB-1a* pollen normally produces ears segregating large and small seeds. The large seeds are produced when the hyperploid sperm fuses with the polar nuclei and the deficient sperm fertilizes the egg nucleus while small seeds result from the reverse combination. In the above cross it was found that the viviparous seeds appeared among the large seeds while none of the small seeds germinated prematurely. Since only the large seeds germinate prematurely it appears that the genetic constitution of the embryo determines premature germination in this mutant. In this respect vp_8 is similar to vp_1, vp_5 and *ps*.

A series of testcrosses were made involving the translocation *T1-7c* and the genes *br* and/or bm_2. The resulting data are summarized in table 14. The break point of *T1-7c* is located about .34 of the distance from the centromere out on the long arm of chromosome one (LONGLEY unpublished). Thus vp_8 is in the outer $\frac{2}{3}$ or this arm about 34.3 crossover units from *T1-7c* and 10.4 from bm_2.

TABLE 15

Test cross data involving gl_1 *and* vp_9

F_1 Parent	Parental combinations		Recombinations		Totals	Percent recombinations
	+ *vp*	*gl* +	++	*gl vp*		
51-2785-9	121	106	7	5	239	5.0
51-2785-14	113	89	16	12	230	12.2
Totals	234	195	23	17	469	8.5

TABLE 16

Test cross progenies of $\frac{wx\ +\ +}{wx\ +\ +} \times \frac{+\ T7\ -\ 9a\ +}{wx\ +\ vp_9}$

F_1 Parent	Parental combinations		Recombinations					Totals	Percent recombinations	
			Region 1		Region 2		Region 1 & 2		Region 1	Region 2
51-2782-15	89	84	7	5	17	13	0 0	215	4.8	12.0
51-2782-10	54	73	4	4	18	34	0 2	189	5.3	28.6
Totals	143	157	11	9	35	47	0 2	404	5.4	20.8

Viviparous-9

A self pollinated ear of a plant heterozygous for translocation *T7-9a*, *wx* and vp_9 revealed linkage between *wx* and vp_9. Since these genes are not linked in the absence of the translocation the linkage in its presence indicates that vp_9 is in chromosome seven. Testcrosses involving *T7-9a* and those with gl_1 have established this. The data from these crosses are found in tables 15 and 16.

The break point of *T7-9a* in chromosome seven is indicated to be L.29 by LONGLEY (unpublished). The position of this break is fairly close to that given for *T2-7b* by ANDERSON and RANDOLPH (1945) which they placed at .2+. LONGLEY (unpublished) has placed the break of this translocation in the long arm of seven at .12. ANDERSON and RANDOLPH (1945) found the break point of *T2-7b* to be to the left of *ra* about 1.1 crossover units which is to the left of gl_1 3.7 units (EMERSON, BEADLE and FRASER 1935). Since the break points of *T2-7b* and *T7-9a* in seven are approximately the same it is very likely that *T7-9a* is to the left of gl_1. Therefore, the most likely order is *T7-9a* − gl_1 − vp_9 with about 20.8% crossing over between *T7-9a* and vp_9 and 8.5% between gl_1 and vp_9.

Pink scutellum

DR. SPRAGUE (unpublished) had indicated the probable linkage relations of this gene to be *ps* − *pr* − gl_8. Data from a modified testcross involving these three genes are given in table 17. Further tests involving only *pr* and *ps* were made. The data from these tests combined with those for this region from table 17 are shown in ta-

TABLE 17

Test cross progeny of $\frac{+\ +\ gl_8}{+\ +\ gl_8} \times \frac{ps\ pr\ gl_8}{+\ +\ +}$

F_1 Parent	Parental combinations	Recombinations			Totals	Percent recombinations	
		Region 1	Region 2	Region 1 & 2		Region 1	Region 2
50-1539-6	79 83	9 19	2 0	0 0	192	14.6	1.0

TABLE 18

Test cross data involving pr *and* ps

F_1 Parent	Total parental combinations	Total recombinations	Totals	Percent recombinations
50-1539-9*	74	30	104	28.8
50-1539-16†	37	11	48	22.9
50-1539-6†	164	28	192	14.6
Totals	275	69	344	20.1

* *pr* and *ps* in repulsion.
† *pr* and *ps* in coupling.

TABLE 19

Test cross progeny of $\frac{+\ +\ wx}{+\ +\ wx} \times \frac{+\ T5\ -\ 9a\ wx}{ps\ +\ +}$

F_1 Parent	Parental combination	Recombinations			Totals	Percent recombinations	
		Region 1	Region 2	Region 1 & 2		Region 1	Region 2
62-804-9	52 41	33 53	0 1	0 1	181	48.1	1.1

ble 18. The results of a testcross involving *wx T5-9a* are given in table 19. The available linkage data from tables 17–18 would indicate the following order for the three genes involved *ps* − 20.1 − *pr* − 1.0 − gl_8. Because of the small numbers neither the order nor position of these genes is certain at this time. Neither can the direction of this linkage map upon the chromosome be determined from these data. The crossover data with *T5-9a* given in table 19 indicate very little if any linkage between the translocation and *ps*. Since LONGLEY (unpublished) has placed the break point of this translocation in the long arm of five at .80, it is very likely that *ps* is to the left of this translocation.

White-3

An F_2 ratio involving w_3 and *su T2-4c* showed linkage with *su*. Since these genes are not linked in the absence of the translocation the linkage in its presence indicates that w_3 is in chromosome two. Further crosses are being made to place this gene with respect to other genes on this chromosome.

Discussion

It has been demonstrated by the embryo culture experiments of LaRue (1936, 1952) and Haagen-Smit, *et al.* (1945) that the immature corn embryo is capable of germinating soon after pollination if removed from the environment of the developing seed. Thus there appears to be some mechanism present in the immature caryopsis which prevents the germination of the embryo during its development. It is this mechanism which is interrupted by the viviparous mutants that have been described in this paper.

The exact nature of the developmental dormancy in maize is not known but the presence of at least eight mutants that affect it would suggest that many factors are involved. It would also seem to be somehow closely related to other phases of plant development since all of these mutants have been demonstrated to affect other mechanisms besides those controlling dormancy. Thus, *vp*$_1$ interrupts pigment formation in the aleurone, *vp*$_5$, *vp*$_9$, *ps* and *w*$_3$ interfere with carotenoid development in the endosperm and seedling as well as preventing chlorophyll formation, *vp*$_2$ besides affecting the endosperm and seedling in a way similar to the previous four genes also reduces the size of the seed, *vp*$_6$ produces necrotic seedlings and *vp*$_8$ produces dwarfed plants.

As deviations are found in the development of the plant insight can be gained as to its normal development. The evidence suggests that the embryos of at least four of the viviparous mutants (*vp*$_1$, *vp*$_8$, this paper; *ps*, Sprague 1936; and *vp*$_5$, Robertson 1952) are capable of germinating in an environment which inhibits the germination of normal embryos. These mutants should be useful tools in the search for the normal mechanism of developmental dormancy.

The Class Two viviparous mutants *vp*$_2$, *vp*$_5$, *vp*$_9$, *ps* and *w*$_3$ probably belong to a larger group of mutants all of which have in common an altered carotenoid synthesis in the endosperm and an altered carotenoid and chlorophyll synthesis in the seedling. If these criteria are used as the primary means of classification then three sub-groups can be recognized: 1) those described in this paper which have pale yellow seeds and albino viviparous seedlings, 2) those which show little if any tendency to germinate prematurely but are otherwise similar to those of Group One, and 3) those which are similar to Group Two in having pale yellow seeds which do not germinate prematurely but differ in that they produce pale green seedlings which die at an early age. Mutants of Group Two have been described by Everett (1949) and Tulpule (1954). The mutants of these workers along with one new Group Two mutant and two of Group Three have been brought together for genetic studies which are proceeding at the present time. The results of these studies will be the subject of a later paper.

Summary

Eight viviparous mutants of maize are described.

A brief description is given of how translocations between chromosomes of the basic set, translocations between a B-type chromosome and one of the basic set, and modified testcrosses were used in locating these mutants.

The following genes were located as to chromosome: *vp*$_1$, chromosome 3; *vp*$_2$,

chromosome 5; *vp*$_5$, chromosome 1; *vp*$_8$, chromosome 1; *vp*$_9$, chromosome 7; *ps*, chromosome 5; and *w*$_3$, chromosome 2.

The known linkage values with genes of their respective chromosomes are given for the above mutants.

The relationship of the viviparous mutants to the normal dormancy mechanism in the maize caryopsis is briefly discussed.

The relationship of *vp*$_2$, *vp*$_5$, *vp*$_9$, *ps* and *w*$_3$ to a larger class of mutants distinguished by an altered carotenoid synthesis in the endosperm and an altered carotenoid and chlorophyll synthesis in the seedling is considered.

ACKNOWLEDGMENT

The author wishes to take this opportunity to thank DR. E. G. ANDERSON for suggesting this problem and for his helpful advice and encouragement; DR. LONGLEY and DR. BANDURSKI for generously permitting the inclusion of unpublished data; DR. PATTERSON for making many crosses for me while I was hospitalized for an ill-timed emergency appendectomy; and DRS. SPRAGUE, BURNHAM, and PHINNEY for supplying viviparous stocks.

LITERATURE CITED

ANDERSON, E. G., 1938 Translocations in maize involving chromosome 9. Genetics **23:** 307–313.
1941 Translocations in maize involving the short arm of chromosome 1. Genetics **26:** 452–459.
1952 A chromosomal technique used in biochemical studies on the developing endosperm of maize. Agron. J. **44:** 560–561.

ANDERSON, E. G., and R. A. BRINK, 1940 Translocations in maize involving chromosome 3. Genetics **25:** 299–309.

ANDERSON, E. G., and L. F. RANDOLPH, 1945 Location of the centromeres on the linkage maps of maize. Genetics **30:** 518–526.

BURNHAM, C. R., 1935 (As cited in) A summary of linkage studies in maize. Cornell Univ. Agr. Exp. Sta. Memoir **180:** 1–83.

DEMEREC, M., 1923 Inheritance of white seedlings in maize. Genetics **8:** 561–593.

EMERSON, R. A., G. W. BEADLE, and A. C. FRASER 1935 A summary of linkage studies in maize. Cornell Univ. Agr. Exp. Sta. Memoir **180:** 1–83.

EVERETT, H. L. 1949 A genic series controlling chloroplast pigments' production in diploid *Zea mays* L. Proc. Nat. Acad. Sci. U. S. **35:** 628–634.

EYSTER, W. H., 1924a A primitive sporophyte in maize. Am. J. Botany **11:** 7–14.
1924b A second factor for primitive sporophyte in maize. Am. Naturalist **58:** 436–439.
1931a Heritable characters of maize. XLII. Reduced endosperm. J. Heredity **22:** 251–252.
1931b Vivipary in maize. Genetics **16:** 574–590.

HAAGEN-SMIT, A. J., R. SIU, and G. WILSON, 1945 A method for culturing of excised, immature corn embryos in vitro. Science **101:** 234.

KOSKI, V. M., and J. H. C. SMITH, 1951 Chlorophyll formation in a mutant, white-seedling-3. Arch. Biochem. and Biophys. **34:** 189–195.

LARUE, C. D., 1936 Growth of plant embryos in culture. Bull. Tor. Bot. Club **63:** 365–382.
1952 Growth of the scutellum of maize in culture. Science **115:** 315.

LINDSTROM, E. W., 1923 Heritable characters of maize: XIII. Endosperm defects—sweet defective and flint defective. J. Heredity **14:** 125–135.
1924 Complementary genes for chlorophyll development in maize and their linkage relations. Genetics **9:** 305–326.

MCCLINTOCK, BARBARA, 1938 The production of homozygous deficient tissues with mutant characteristics by means of the aberrant mitotic behavior of ring-shaped chromosomes. Genetics **23:** 315–376.

1941 The association of mutant with homozygous deficiencies in *Zea mays*. Genetics **26:** 542–571.

MANGELSDORF, P. C., 1923 The inheritance of defective seeds in maize. J. Heredity **14:** 119–125.

1926 The genetic and morphology of some endosperm characters in maize. Conn. Agr. Exp. Bull. **279:** 513–614.

1930 The inheritance of dormancy and premature germination in maize. Genetics **15;** 462–494.

RHOADES, M. M., 1936 A cytogenetic study of a chromosome fragment in maize. Genetics **21:** 491–502.

ROBERTSON, D. S., 1951 The genotype of the endosperm and embryo as it influences vivipary in maize. Proc. Nat. Acad. Sci. U. S. **38:** 580–583.

ROMAN, H., 1947 Mitotic nondisjunction in the case of interchanges involving the B-type chromosomes in maize. Genetics **32:** 391–409.

ROMAN, H., and A. ULLSTRUP, 1951 The use of A and B translocations to locate genes in maize. Agron. J. **43:** 450–454.

SPRAGUE, C. F., 1929 Hetero-fertilization in maize. Science **69:** 526–527.

1932 The nature and extent of heterofertilization in maize. Genetics **17:** 358–368.

1936 The relation of moisture content and time of harvest to germination of immature corn. J. Am. Soc. Agron. **28:** 472–478.

1945 Genetic investigation with corn. Tenth annual report. Iowa Corn Research Institute pp. 49–50.

TULPULE, S. H., 1954 A study of pleiotropic genes in maize. Am. J. Botany **41:** 294–301.

GENETICS SOCIETY OF AMERICA

EXECUTIVE COMMITTEE

President, P. C. MANGELSDORF — *Vice-President*, R. E. CLELAND

Past Presidents, ROY E. CLAUSEN, J. T. PATTERSON

Secretary, C. P. OLIVER
University of Texas
Austin 12, Texas

Treasurer, N. H. GILES, JR.
Yale University
New Haven, Connecticut

"The Genetics Society of America is organized to provide facilities for association and conference among students of heredity and for the encouragement of close relationship between workers in genetics and those in related subjects. All persons actively interested in any field of genetics shall be eligible to active membership. Candidates for membership must be recommended by two members of the Society. Members shall be elected by the Executive Committee."—Constitution, Article I.

Any one interested in joining the Society should write to the Secretary.

The Genetics Society publishes annually the Records of the Society containing the program and abstracts of the Annual Meeting and others items of interest to members, including a membership list at periodic intervals. Total membership of the Society was 979 on September 10, 1954. Copies of the Records may be obtained from the Treasurer for $1.00. The Genetics Society has a representative on the Editorial Board of GENETICS, Tracy M. Sonneborn.

SUSTAINING MEMBERS

The Genetics Society of America established in 1949 a new type of membership, Sustaining Members. The constitution provides that "All organizations interested in any field of genetics shall be eligible to sustaining membership." The dues for sustaining members are $50.00 a year. Money contributed by sustaining members is not used for running expenses of the Society, but is used for special purposes as decided by the Executive Committee. The Executive Committee at a meeting on September 7, 1954, voted unanimously that $500 from the sustaining membership account be allocated to GENETICS toward its publication costs.

The following organizations became sustaining members of the Society in the years indicated:

Associated Seed Growers
New Haven, Conn.—1950

Bear Hybrids Corn Co.
Decatur, Ill.—1950

W. Atlee Burpee Co.
Philadelphia 32, Pa.—1950

DeKalb Hybrid Seed Co.
DeKalb, Ill.—1949

Ferry-Morse Seed Co.
Detroit 31, Mich.—1949

Funk Brothers Seed Co.
Bloomington, Ill.—1950

Kimber Farms
Niles, California—1953

Moews Seed Co.
Granville, Ill.—1949

Nichols Poultry Farm
Kingston, N. H.—1950

Pioneer Hi-Bred Corn Co.
Johnston, Iowa—1949

This page is donated by GENETICS to the GENETICS SOCIETY OF AMERICA. The society and the journal are separate organizations with a common bond of interest in furthering the science of heredity.

INFORMATION FOR CONTRIBUTORS

Contributions to GENETICS may be in the field of genetics proper, or in any scientific field, if of such a character as to be of primary genetic interest.

The length of manuscripts will be limited to twenty printed pages, including tables and illustrations. Longer papers may be published by special vote of the Editorial Board and, ordinarily, only if the author agrees to bear the cost of the additional pages. In case unusually extensive tabular material cannot be printed, it will be kept on file on request, provided two copies are furnished by the author.

The *Galton and Mendel Memorial Fund* established in 1923 from donations of biologists and other persons interested in the progress of biological discovery is available to be applied toward the cost of reproducing illustrations and of printing expensive tables and formulae.

Authors should submit two copies of their manuscripts to the Editors. Manuscripts should conform to the general usage in GENETICS, particularly in regard to references to literature, arrangement of "Literature Cited" and inclusion of a "Summary". Excessive footnotes should be avoided. Legends for figures and plates should be typewritten on separate pages. Tables should be on separate pages. Material for figures should be original drawings (clear photographs usually are satisfactory for line drawings) and should be of a size permitting slight reduction on the printed page. Photographs and drawings substantially larger than a typewritten page should not be sent; they are difficult to handle and are likely to be damaged in the mail. Reviewing of the manuscript is facilitated if photographic copies of the figures are included.

The manuscript of a published paper will not be returned unless the author so requests.

Galley proofs will be sent to authors but page proofs will be sent only in exceptional cases. Authors should leave forwarding directions whenever they are away from the address sent with the manuscript or should make other arrangements to have the galleys corrected promptly.

Reprints should be ordered directly from the printer at the time galley proof is returned to the Editors. The author will be billed in accordance with the schedule of charges appearing on the reprint order blank. Copies of the schedule are available from Genetics, Inc., Mt. Royal and Guilford Avenues, Baltimore 2, Md.

Manuscripts and editorial correspondence should be addressed to the Editors of GENETICS, Genetics Building, University of Wisconsin, Madison 6, Wisconsin.

CONTENTS OF PRECEDING ISSUES

MARCH, 1955

KEMPTHORNE, OSCAR, The theoretical values of correlations between relatives in random mating populations.

KEMPTHORNE, OSCAR, The correlation between relatives in a simple autotetraploid population.

HALL, B. M., Genetic analysis of interspecific hybrids in the genus Bromus, section Ceratochloa.

SWANSON, C. P., Relative effects of qualitatively different ionizing radiations on the production of chromatid aberrations in air and in nitrogen.

JAMES, ALLEN P., A genetic analysis of sectoring in ultraviolet-induced variant colonies of yeast.

MENZEL, MARGARET Y., A cytological method for genome analysis in Gossypium.

HINTON, TAYLOR, The genetic basis of a nutritional requirement in Drosophila.

RHYNE, CLAUDE L., The inheritance of yellow-green, a possible mutation in cotton.

SLATIS, HERMAN M., A reconsideration of the brown-dominant position effect.

GLASS, BENTLEY, A comparative study of induced mutation in the oocytes and spermatozoa of *Drosophila melanogaster*. I. Translocations and inversions.

PLAINE, HENRY L., The effect of oxygen and of hydrogen peroxide on the action of a specific gene and on tumor induction in *Drosophila melanogaster*.

GLASS, BENTLEY, A comparative study of induced mutation in the oocytes and spermatozoa of *Drosophila melanogaster*. II. Deficiencies and minutes.

MAY, 1955

YOON, CHAI H., Homeostasis associated with heterozygosity in the genetics of time of vaginal opening in the house mouse.

HORNER, T. W., and O. KEMPTHORNE, The components of variance and the correlations between relatives in symmetrical random mating populations.

BUTZEL, HENRY M., JR., Mating type mutations in variety 1 of *Paramecium aurelia*, and their bearing upon the problem of mating type determinations.

ERK, FRANK C., Competition between chromosomal aberrations associated with Curly and their wild type homologues in laboratory populations of *Drosophila melanogaster*.

STERN, CURT, and MASUO KODANI, Studies on the position effect at the cubitus interruptus locus of *Drosophila melanogaster*.

LEFEVRE, G., JR., Mutation isoalleles at the yellow locus in *Drosophila melanogaster*.

NANNEY, DAVID L., and PATRICIA A. CAUGHEY, An unstable nuclear condition in *Tetrahymena pyriformis*.

LEVINE, R. P., and ELIZABETH E. LEVINE, Variable crossing over arising in different strains of *Drosophila pseudoobscura*.

YAMAMOTO, TOKI-O, Progeny of artificially induced sex-reversals of male genotype (XY) in the medaka (*Oryzias latipes*) with special reference to YY-male.

JULY, 1955

SCHALLER, C. W., and F. N. BRIGGS, Inheritance of resistance to mildew, *Erysiphe graminis hordei*, in the barley variety, Black Russian

JANICK, JULES, AND E. C. STEVENSON, Genetics of the monoecious character in spinach.

ATWOOD, K. C., and FRANK MUKAI, Nuclear distribution in conidia of Neurospora heterokaryons.

HEXTER, WILLIAM M., A population analysis of heterozygote frequencies in Drosophila.

DESCHNER, ELEANOR, and A. H. SPARROW, Chromosome rejoining capacity with respect to breakage sensitivity to X-rays and thermal neutrons.

SAGER, RUTH, Inheritance in the green alga *Chlamydomonas reinhardi*.

KING, R. C., and EUNICE M. WOOD, Sex-linked lethal mutations induced by thermal neutrons in male and female *Drosophila melanogaster*.

ANDERSON, E. G., H. H. KRAMER, and A. E. LONGLEY, Translocations in maize involving chromosome 4.

HAWTHORNE, DONALD C., The use of linear asci for chromosome mapping in Saccharomyces.

MOREJOHN, G. VICTOR, Plumage color allelism in the red jungle fowl (*Gallus gallus*) and related domestic forms.

ANDERSON, E. G., H. H. KRAMER, and A. E. LONGLEY, Translocations in maize involving chromosome 6.

BOYLE, W. S., and A. H. HOLMGREN, A cytogenetic study of natural and controlled hybrids between *Agropyron trachycaulum* and *Hordeum jubatum*.

ROMAN, HERSCHEL, MARCIA M. PHILLIPS, and STANLEY M. SANDS, Studies of polyploid Saccharomyces. I. Tetraploid segregation.

SINGLE NUMBERS $1.50 ANNUAL SUBSCRIPTION $8.00

obtainable from

GENETICS, INC., BUSINESS OFFICE, MT. ROYAL AND GUILFORD AVES.,
BALTIMORE 2, MARYLAND